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BEARING ON HEREDITY AND VARIATION

Volume 24 NOVEMBER, 1939 Number 6

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A SPONTANEOUS SOMATIC EXCHANGE BETWEEN NON-HOMOLOGOUS CHROMOSOMES IN DROSOPHILA MELANOGASTER

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Received May 31, 1939

INTRODUCTION

THE first kind of exchange between chromosomes to be discovered in Drosophila was crossing over, a term which when used without qualification still stands for exchange between homologous chromatids at identical loci in the four strand stage of meiosis in the female.

Spontaneous somatic crossing over has subsequently been observed resulting from exchange between homologous chromosomes of cells of the soma in females and males, and also of gonial cells, that is of cells in all of which nuclear divisions are mitotic. The frequencies of all of these kinds of exchange is known to be affected by one or more of such agents as X-rays, temperature and the presence of Minute mutations.

Exchange between chromosomes at non-homologous loci may readily be induced by X-raying males or females either when mature or at different stages of development. The exchanges result in different kinds of aberrations; they may be interchromosomal translocations (between arms of different chromsomes) or intrachromosomal translocations (a), between different arms of the same chromosome, called "eucentric inversions" by CATCHESIDE (1938), (b), within one arm, that is inversions, or (c), transpositions which involve at least three breaks in one chromosome.

The nuclei in which observed translocations have been induced have been those of the germ track. Sperms, among mature and immature germ cells of both sexes, are most readily affected.

Spontaneous aberrations involving non-homologous loci are extremely rare in *D. melanogaster*. Pale translocation (BRIDGES 1923) arose without treatment and had its origin in germinal cells since it is transmitted. Similarly Translocation E (2; 3) (STURTEVANT and DOZBHANSKY 1930) almost certainly arose spontaneously.

Spontaneous translocations in the soma, due to exchange between chromosomes at non-homologous loci, may be still more rare. There has been some opportunity to detect them by a method suggested by STERN (1939). In flies heterozygous for an inversion or for a closed X chromosome, single crossing over leads to the formation of some nuclei of unbalanced constitution. STERN believes that some somatic cells containing unbal-

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anced nuclei may survive in the soma and identifies them with very small patches found to contain often only one short, fine seta. He believes that if somatic non-homologous exchange occurred in flies of normal chromosome constitution, the altered constitution which results also from exchange in cells heterozygous for a translocation would be similarly detectable and would have been observed unless they occurred only with extremely low frequency. He also examined flies genetically marked in a manner that would certainly have disclosed recognizable spots if somatic non-homologous translocation had occurred with a frequency equal to that of somatic crossing over. But neither has this possible source of evidence yielded any results.

A very favorable opportunity for detection of spontaneous translocation in one kind of somatic tissue is afforded by all experiments involving the inspection of salivary glands. In the very numerous X-ray experiments that have been carried out, a spontaneous translocation in a cell destined to contribute to the salivary gland might have been detected in the glands of any larva whether it had developed from sperm affected or unaffected by the X-ray treatment. A larval gland in which such a spontaneous translocation had occurred would contain it in some of the nuclei. The larvae from affected sperm would contain, in addition, the induced translocation in all of their nuclei. No instance of a spontaneous somatic translocation in salivary glands seems to have been reported in the literature, but recently one has been found in a mutant stock of *Drosophila melanogaster*

NEW OBSERVATIONS

Salivary glands of stock B^{36j} were being examined for a possible visible alteration of the bands near the Bar locus which might be correlated with the slightly bar allele of *B*, as originally found in a y^2 male. No such change has so far been detected but the large autosomes of two (possibly three) cells of one gland show a translocation, T(2; 3)39b, not present in the other nuclei.

The exceptional nuclei are heterozygous for a reciprocal translocation between the right arm of the second chromosome and the right arm of the third (figure 1a, b). The breaks occur in regions 57B in the second and 98C in the third chromosome (following BRIDGES' system), points that are not far distant from the distal ends of the chromosomes. In both nuclei the unbroken chromosomes are synapsed with their fractional homologues at their distal ends. In one nucleus (figure 1a) the proximal fragmented 3R is synapsed with the normal 3R. The proximal fragmented 2R and the entire 2R remain unsynapsed for a long distance to the right of the break

and as far as they can be traced toward the chromocenter. In the other nucleus (figure 1b) the entire and the broken 2R are synapsed and the 3R chromsomes are largely unsynapsed.

In the same gland the other nuclei in which the chromosomes are sufficiently spread have been examined. In 11 nuclei the distal sections of 2R and 3R show clearly and are not in any way associated with each other. In 10 other nuclei either one or the other of the two distal ends of the two chromosomes in question is entirely free. Special study was made of the

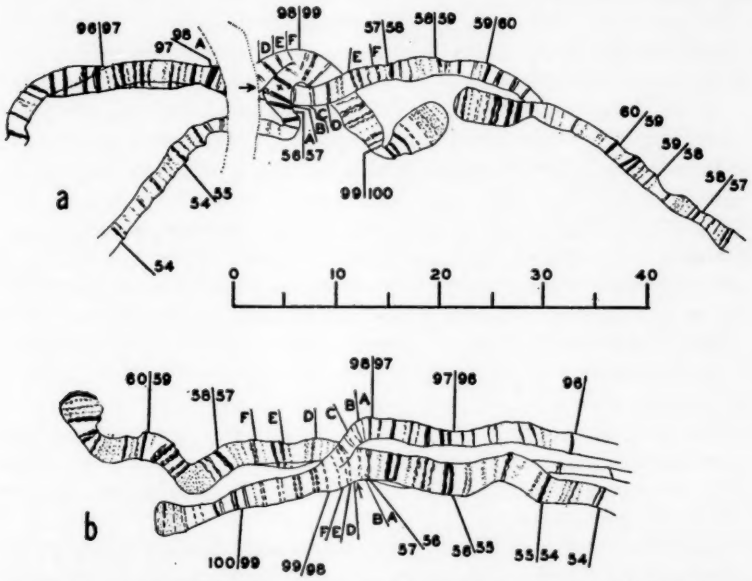

FIGURE 1.—Salivary chromosomes of two nuclei containing a somatic translocation, T(2; 3) 39b, between 2R and 3R. The region of one of the break-points is indicated by arrows. The scale (40 micra) indicates the magnification used in making the drawings.

more confused nuclei in which synapsis was not complete. In one of them two chromosomes are unsynapsed from a point near to the distal end of 2R, but the bands match in the haploid parts showing that there is no translocation with another chromosome. In two others haploid sections are not associated with a 2 to 3 translocation. One nucleus showing haploid strands has not been deciphered.

The observations are sufficient to show that a translocation occurred in the last or in a very late division in the nucleus of a somatic cell before the full number of cells of the salivary gland was attained.

DISCUSSION

The finding, in the soma, of a spontaneous occurrence of translocation, long known as a frequent kind of aberration induced in germ cells, leads to consideration of various effects on chromosomal behavior that have been obtained experimentally.

The extensive studies that have been made on the effects of external agents on interchange between chromosomes have shown that there are marked differences in the magnitude of effects of external agents on different kinds of exchange.

X-rays, high and sometimes low temperature and age, the presence of Minute mutations and sometimes the presence of the Y chromosome have been found to affect exchange between chromosomes at homologous loci in germ cells or somatic cells, usually causing increase in frequency of exchange especially near spindle fiber regions. Minutes have been found to increase somatic crossing over but when combined with high temperature there is a decrease (STERN and RENTSCHLER 1936). Induction by high temperature (WHITTINGHILL 1937) of exchanges between homologous chromosomes in the germ track of larval males produced no abnormal effect upon subsequent crossing over of the treated chromosome or viability of homozygotes, the usual properties of translocations. The same was found to be true of the majority of exchanges between homologous chromosomes obtained by PATTERSON and SUCHE (1934) from X-ray treatment of male larvae.

The order of magnitude of all of these several effects is not greater than that of temperature on chemical reactions. The processes affected, which include crossing over at meiosis, occur spontaneously with different but observable frequencies.

In addition to these effects X-rays cause random breaks, recoverable in translocations, which involve reattachment of broken ends at non-homologous loci.

Two results may be especially noted in connection with translocations or exchanges at non-homologous loci in *Drosophila melanogaster*. First, exchanges at non-homologous loci appear to be the least frequent of spontaneous exchanges, but they are the exchanges which are induced by X-rays, at least in germ cells, with high frequency increasing with the dosage. The rate of breakage may be so high that broken chromosomes recombined into viable combinations are present in as much as 40 percent of functional sperm (CATCHESIDE 1938; BAUER, DEMEREC and KAUFMANN 1938).

Second, in some X-ray experiments (MULLER 1925) interference was found to be but slightly, if at all, affected by the X-ray treatment, which

increased crossing over especially near the spindle fiber attachment. BAUER, DEMEREC and KAUFMANN point out that in contrast to the dependence of the position of one break upon the position of a second simultaneous break at crossing over, breaks induced by X-rays and found in translocations occur at random in euchromatic regions, that is the position of one break is independent of the position of another break in the same chromosome.

It appears then that X-rays may have two different, and both to some extent regionally differential, effects; they affect to a relatively slight extent (among other processes) the rate of crossing over, which is a natural ("spontaneous") process, depending on the properties and mechanism of the chromosomes and occurring regularly in all females; and X-rays also cause a high percentage of breaks in the chromosomes, many of which may be recovered in translocations but which are very rarely found in untreated material. The mode of reattachment of broken ends has not been solved even by recent critical experiments.

The possible importance of the telophase pattern of pairing of chromosomes in an understanding of aberrations was suggested by PAINTER (1934 and 1935). It has been emphasized also by DOBZHANSKY (1936) who found evidence from secondary spermatogonial nuclei of *Drosophila pseudoobscura* that if the telophase distribution is retained in young resting nuclei it must be more or less preserved during the entire interphase.

METZ (1916), from his extensive cytological studies of somatic, oogonial and spermatogonial cells of Drosophila and other Diptera, thought it highly probable that the chromosomes retain the telophase arrangement during the transformations in the resting nucleus.

MAKINO (1938) presents another kind of evidence in regard to the condition of the chromosomes in the resting stage. He has found that the definitive nuclei which are developed before larval stages, in *Drosophila virilis*, show in many tissues of the larva the same grouping of the haploid number of chromosomes that is found in salivary glands and Malphigian tubes. This involves close synapsis of homologues and attachment of one end of each of the chromosomes to a common center. He regards it as a condition of permanent resting stage. FROLOVA (1938) has made similar observations on various tissues of larvae of *melanogaster*, *virilis* and three other species of Drosophila.

If the telophase pattern persists through all stages of the nuclear cycle it is a comparatively constant factor in the origin of translocations.

In order to induce translocations in somatic cells contributing to the salivary glands, where rearrangements resulting from breaks might readily be observed, the embryo must be treated before the eighteenth hour of

development when the definitive number of cells of the salivary gland has been attained (POULSON 1937).

SUMMARY

An interchange of sections between the right arm of chromosome 2 and the right arm of chromosome 3 was found in two of the nuclei of a salivary gland of *Drosophila melanogaster*. Thus interchange between non-homologous chromosomes may, like mutation, elimination and regular and somatic crossing over, occur spontaneously in somatic nuclei of this species, though probably with relatively very low frequency.

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INTROGRESSIVE HYBRIDIZATION IN A NATURAL POPULATION OF TRADESCANTIA

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Received June 21, 1939

INTRODUCTION

DETAILED analyses of populations containing natural hybrids have been reported recently. ANDERSON (1936) has suggested a method for expressing quantitatively the extent of hybridity of a population and for comparing various populations, and this method has been applied to various genera such as Tradescantia (ANDERSON 1936), Solidago (GOODWIN 1937), and Iris (RILEY 1938, 1939) The present paper reports a morphological and cytological study of a population of Tradescantia growing along a railroad right-of-way about fifteen miles east of the center of New Orleans, Louisiana. This population was more complex than most of the populations of that genus reported by ANDERSON and HUBRICHT (1938) for it involved three species of which two were tetraploids and the third a diploid.

MATERIALS AND METHODS

The plants used for this investigation were growing along the side of the railroad track at the intersection of the Louisville and Nashville R. R. and the Chef Menteur highway (U.S. route 90) at Michaud, Louisiana, near signal tower number 7934. During the spring of 1936, the author (RILEY 1937) made a preliminary survey of the region, examining 52 plants, and this study was continued in 1937 using 93 plants and several additional characters. Ten characters were recorded for most of the plants, but on some it was not possible to get a chromosome count, on others no fertility records could be obtained, and on a few both kinds of data were unrecorded. Plants upon which information was incomplete were marked and the missing data were obtained when they bloomed again in the spring of 1938. Herbarium specimens were made for all 93 plants.

A more exact picture of the location of these plants and of their spatial relationships to one another can be obtained from figure 1. The railroad embankment begins to slope downward about two meters from the track. A number of plants were located just at the upper edge of this embankment and most of the rest were found on the slope. Plant 29 was growing near the tracks and plants 9 through 15 were in the level ground at the

[1] Contribution from Newcomb College, Tulane University, New Orleans, Louisiana. This study was aided in part by a grant from the New Orleans Academy of Science.

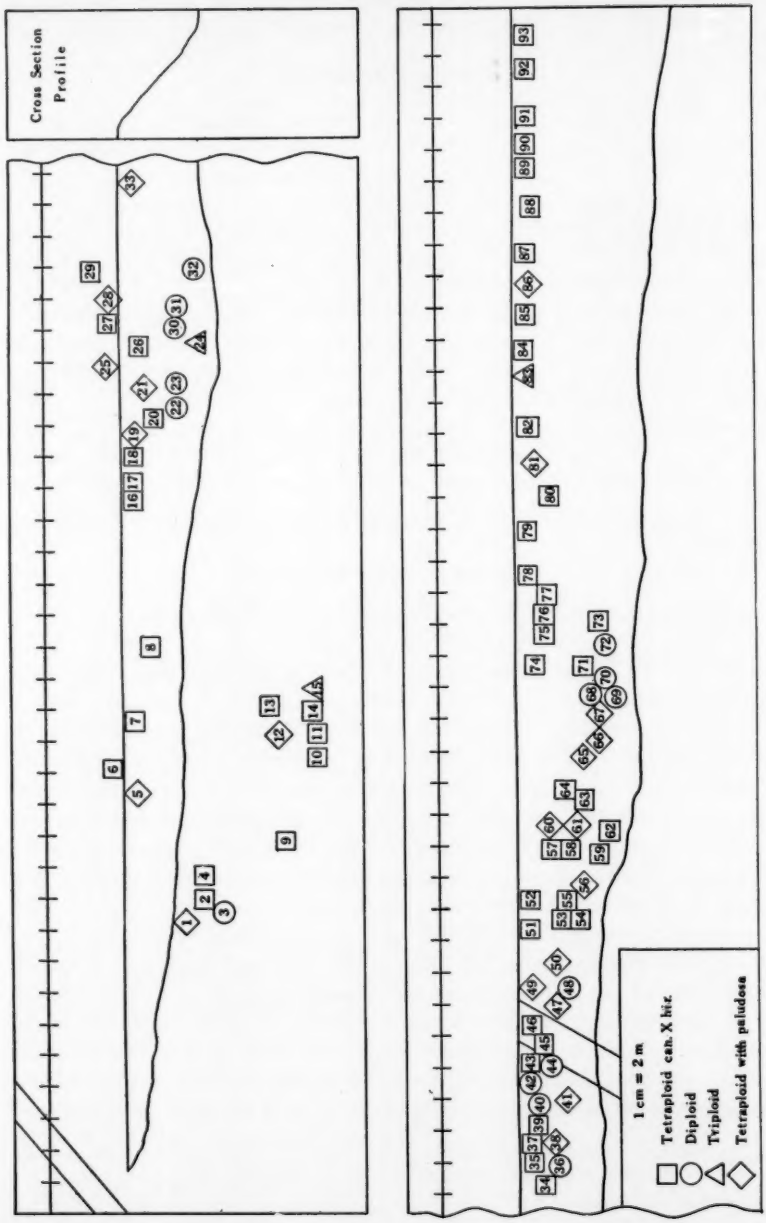


FIGURE 1.—*(See opposite page for legend.)*

bottom of the embankment. About 20 meters from the railroad track, the land rises again into a fairly dense woods and there is a path to the woods from the railroad embankment starting at the location of plants 43 and 49. These details of the topography of the region are important as they affect the distribution of the plants. During the spring, the location is a sunny one and the plants are at their peak from March through May, but by late June most of their leaves have disappeared and the flower stalks are well advanced; as the summer progresses, other plants grow up and shade the Tradescantias which are at that time located only with great difficulty.

As has been stated previously, the plants at this right-of-way appear to exhibit characters of three species. These are *Tradescantia canaliculata* Raf. (=*T. reflexa* Raf.), *T. hirsutiflora* Bush and *T. paludosa* Anders. and Woods. The type locality of *T. paludosa* is near the Gentilly section of New Orleans and the author has collected it in a number of places in southeastern Louisiana where it is ubiquitous. The other two species are known from several places in southeastern Louisiana but uncontaminated specimens were not found in the immediate vicinity of the population studied at Michaud. In all cases where the author has found specimens of *T. hirsutiflora* and *T. canaliculata* in southeastern Louisiana they showed signs in having intercrossed and probably none represented a pure species. Therefore, local plants could not be relied upon with safety as standards of those two species, and the author depended chiefly upon herbarium specimens which were supplied to him by DR. EDGAR ANDERSON who had collected them in localities where they were not intercrossing, and upon descriptions from ANDERSON'S and WOODSON'S (1935) monograph. The ten characters which were selected for study and their appearance in the three species are as follows:

Character	*T. canaliculata*	*T. hirsutiflora*	*T. paludosa*
Distribution of stomata	about equal on upper and lower surfaces	somewhat more numerous on lower surface	very few on upper surface
Shape of leaf	long and narrow	short and broad	short; not so broad; more or less subpetiolar base
Shape of stomata	large with conspicuous cells at ends	large; generally without cells at ends	small

FIGURE 1.—Map of the area discussed in this paper, showing the Louisville and Nashville railroad track at Michaud, Louisiana, the Chef Menteur highway where it crosses the track, part of the embankment and general land at the side of the railroad track, and the location of the 93 plants which were examined. The general contour can be determined from the cross-section profile.

Character	*T. canaliculata*	*T. hirsutiflora*	*T. paludosa*
Number of nodes	7–8; smaller above	2–5; largest above	5–7
Straightness of stem	straight or nearly so	straight	strongly flexed at nodes
Stoutness of stem	stout	somewhat stout	slender; almost delicate above
Size of buds	large	large	small
Color on pedicels	slight purple flush	deep purple	green
Hairiness of pedicels	glabrous	generally hairy	glabrous
Hairiness of sepals	glabrous except for tuft at tip of sepals	hairy	glabrous

OBSERVATIONS

The 93 plants which were studied in this investigation can be divided into the following four groups and as each group presents different problems, they will be discussed separately:

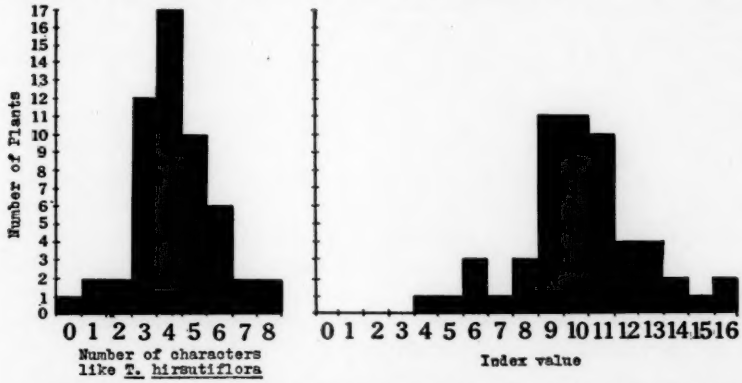
Tetraploids	*T. canaliculata*×*T. hirsutiflora*	54 plants
Diploids	*T. paludosa*	15 plants
Triploids	All three species	3 plants
Tetraploids	All three species	21 plants

Tetraploids—T. canaliculata×*T. hirsutiflora*

Seventy-five of the 93 plants were tetraploids (n = 12), and 54 of these showed no indication of hybridization with *T. paludosa*. It is probable that all 54 plants represent some degree of hybridity between *T. canaliculata* and *T. hirsutiflora* although two plants (nos. 29 and 82) appeared to resemble *T. hirsutiflora* in all ten of the characters which were studied. In straightness of stem and size of buds, *T. canaliculata* and *T. hirsutiflora* do not differ from one another to any measureable extent. They differ, however, in the other eight characters, and each of these eight characters may resemble either of the two species or may be intermediate between them. There are three possibilities for each of the eight characters; if the chance of each possibility can be assumed to be equal to that of any other possibility, and if each character is determined independently of all the other characters, there would be 6561 possible types of tetraploids not involving *T. paludosa*. Of the *canaliculata*×*hirsutiflora* tetraploids which were examined, only two were alike; there are 53 different recombinations represented in these 54 plants. The number of plants with eight, seven, six, etc. characters like *T. hirsutiflora* were tabulated and plotted in figure 2. While this histogram shows a fairly symmetrical distribution of *hirsuti-*

flora-like and non-*hirsutiflora*-like characters, it must be remembered that of the latter class some are like *T. canaliculata* and others are intermediate. If characters which resemble *T. hirsutiflora* are given the value 2, those like *T. canaliculata* are scored 0, and intermediates are given the value 1, and if the total index values of the plants are plotted (figure 3), it is seen that the curve is shifted towards *T. hirsutiflora*. This would appear to indicate that when non-*paludosa* plants of this population originated, there were more specimens of *T. hirsutiflora* than of *T. canaliculata* in the region of hybridization, so that the hybrid types show an introgression of *T. canaliculata* into *T. hirsutiflora*.

These tetraploids ranged in pollen fertility from 47.9 to 97.1 percent. Figure 4 is a scatter diagram in which X represents the percent of fertile



FIGURES 2 and 3.—Figure 2 shows the distribution of the *canaliculata*×*hirsutiflora* tetraploids according to the number of characters like *T. hirsutiflora*, and figure 3 shows the distribution of the same plants according to the index values; for method of determining index values, see text.

pollen and Y the index values of these non-*paludosa* tetraploids. If there is correlation, it should show a curvilinear and not a straight line relationship since plants with high index values and also those with low index values should be more highly fertile than the intermediates. No such correlation appears from the diagram. The value of η_{xy} is 0.4186 and that of η_{yx} is 0.4382. There is no doubt that these plants were all tetraploids. Figure 5 illustrates a first meiotic metaphase of a typical plant showing bivalent and quadrivalent configurations and the normal tetraploid number of 24 chromosomes. Figures 6 and 7 represent metaphases of the haploid microspore division of two other plants and show their tetraploid nature; in addition to the twelve normal chromosomes the plant in figure 7 also shows one small fragment.

Diploids of T. paludosa

Fifteen of the 93 plants resembled *T. paludosa* in most respects and were diploids (n=6). Four were pure *T. paludosa*, but the others seemed to show some influence of *T. hirsutiflora* in that their pedicels took on some of

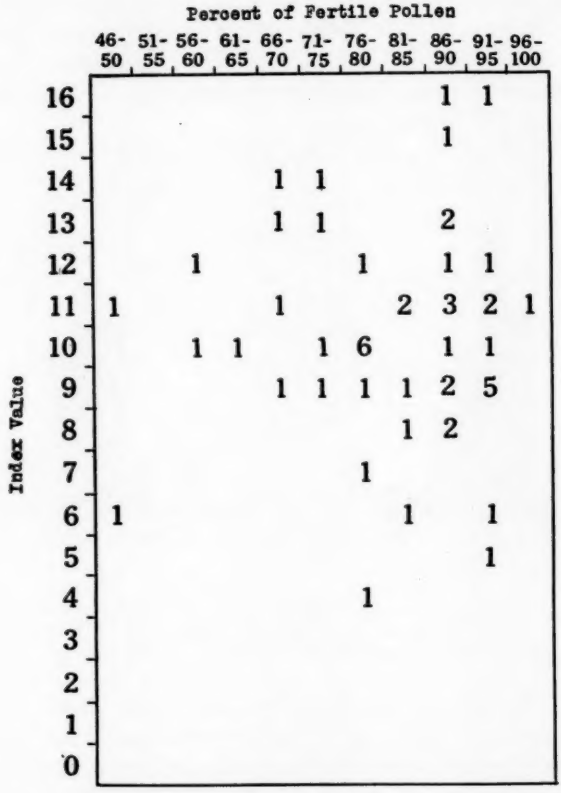
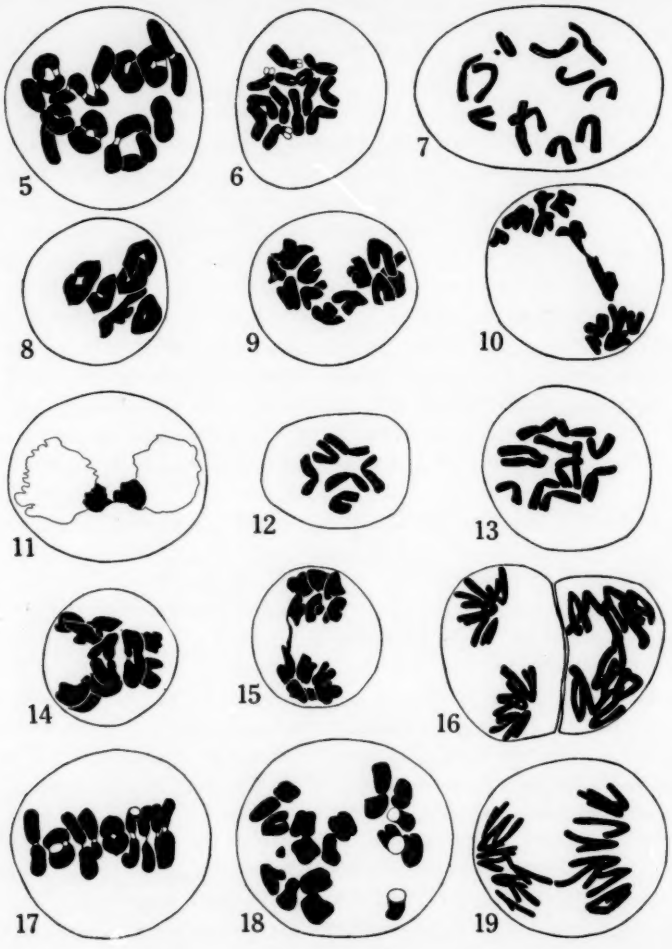


FIGURE 4.—Scatter diagram of 54 *canaliculata*×*hirsutiflora* tetraploid hybrids. Percent of fertile pollen is plotted on the X axis and index value on the Y axis.

the purplish color of the latter species. Two plants showed some influence of both tetraploid species. All 15 plants very closely resembled *T. paludosa*; those that showed influence of either or both tetraploid species showed just a trace of influence as if one of the *paludosa* chromosomes which normally had the constitution *p-p-p-p-p-p-p-p-p-p-p-p* had acquired a gene or two from the tetraploids and was now *p-p-p-p-h-p-p-p-p-p-p* or

perhaps *p-p-h-p-p-p-c-p-p-p-p-p*. In pollen fertility, these plants ranged from 66.7 to 98.9 percent fertile with nine plants over 90 percent fertile and 13 of the 15 over 80 percent. The average is higher than in the case of *canaliculata*×*hirsutiflora* hybrids.

Chromosome behavior in these diploids shows a number of irregularities of different kinds. Plant 68 was typical of *T. paludosa* in every respect, had six pairs of chromosomes and was 95.42 percent fertile. Figure 8 illustrates a metaphase of the first meiotic division in which two ring bivalents are interlocked similar to some of those described by SAX and ANDERSON (1934) in *T. edwardsiana* and which can be found in varying percentages in plants of a number of species of Tradescantia. Out of 114 cells in the first meiotic metaphase of plant 68, 33 (28.95 percent) showed one or more interlocked figures; of these, 25 cells had one pair of interlocked ring bivalents, three had one ring bivalent interlocked with a rod bivalent, one cell had three ring bivalents interlocked together, one cell had two ring bivalents and two rod bivalents all interlocked, two cells had two pairs of interlocked ring bivalents, and one cell had one pair of interlocked ring bivalents and one interlocked ring and rod. None of the ring bivalents had two internodes, so apparently all the interlocking in these cells is proximal, which is in accord with SAX and ANDERSON'S (1934) observations for *T. edwardsiana*. The chiasma frequency was 1.97 per bivalent for the cells that did not include interlocked configurations nor rings of four chromosomes and was 1.96 for the 33 cells with interlocking; this agrees with SAX and ANDERSON'S observations that chromosome interlocking does not decrease the chiasma frequency per bivalent. At anaphase of the first meiotic mitosis, lagging of chromosomes, chromatid bridges and non-disjunction were observed. Figure 9 illustrates an anaphase in which five pairs of homologues have separated while the sixth is just beginning to separate and figures 10 and 11 show chromatid bridges at late anaphase and telophase. Of 75 cells in the anaphase of the first meiotic division, five (6.67 percent) showed chromatid bridges and nine (12 percent) showed non-disjunction with a 5–7 distribution of the chromosomes. MCCLINTOCK (1933, 1938) and SAX (1937) showed that chromatid bridges could be caused by a single crossover within an inversion, and MCCLINTOCK pointed out that cells which contain single bridges at the first division should also have one fragment which did not include an attachment region. However, in SAX'S results no such fragment was detected and many cells of plant 68 that had chromatid bridges did not contain a fragment. That a chromatid bridge interferes with the normal timing of meiosis can be observed from figure 11, where two telophase nuclei have formed except for the homologues which are still connected by the bridge. Metaphase and anaphase of the first division of the haploid microspore nucleus were for the most part



FIGURES 5–19

Camera lucida drawings of aceto-carmine smears of meiotic and microspore divisions of various diploids, triploids and tetraploids of Tradescantia. A Bausch and Lomb microscope was used with a 100× fluorite objective, N. A. 1.30 and a 12.5× ocular, drawn at table level. Magnification ×1725 before reduction; reduced approximately two-thirds.

FIGURE 5.—Metaphase of the first meiotic mitosis of plant 8. This shows the normal tetraploid number of chromosomes with characteristic bivalent and quadrivalent configurations.

FIGURE 6.—Metaphase of the microspore division of the tetraploid plant 77, with the typical number of 12 chromosomes.

normal, but there were some tetraploid microspores among the majority of normal diploid spores. Figure 12 illustrates a normal diploid microspore (n=6) and figure 13 one of the tetraploids (n=12) from the same bud. Out of 172 cells at metaphase, 25 (14.5 percent) were tetraploid and at anaphase 13 cells (10.7 percent) out of 122 were giants. Of 2166 microspores in all stages, including one- and two-nucleate resting cells, 174 (8.03 percent) appeared abnormally large and can be presumed to be tetraploids.

At metaphase of the first meiotic division of plant 48, five cells with interlocked ring bivalents were observed out of fifty. In one, a ring bivalent with two chiasmata was interlocked proximally with another that had three chiasmata and two internodes. In figure 14, four pairs of anaphase chromosomes have almost reached the poles; a fifth pair has barely begun to separate and the sixth bivalent is still in the metaphase condition. A typical chromatid bridge from plant 48 is illustrated in figure 15. Too few figures were observed at anaphase to be of much significance, but they showed that cells with bridges and non-disjunction were not numerous.

No detailed study was made of chromosomal fragments but they were

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FIGURE 7.—Metaphase of the microspore division of the tetraploid plant 34. In addition to the normal number of 12 chromosomes, a fragment is present.

FIGURE 8.—First meiotic metaphase of plant 68. This is a diploid, *T. paludosa*, with six bivalents and shows two interlocked rings.

FIGURE 9.—First meiotic anaphase of plant 68, showing one pair of chromosomes slower to separate than the other five pairs.

FIGURE 10.—Late anaphase of the first meiotic division of plant 68. Five pairs of chromosomes have passed to the poles while in the sixth, both members are tied together by a chromatid bridge.

FIGURE 11.—Telophase of the first meiotic division of plant 68. The members of one pair of chromosomes are connected by a chromatid bridge and have not yet separated. This pair of chromosomes is indicated in black, while the telophase nuclei are shown in outline only.

FIGURE 12.—A normal microspore of the diploid plant 68 with six chromosomes in metaphase.

FIGURE 13.—Metaphase of the microspore division of a tetraploid microspore from the diploid plant 68. This figure shows 12 chromosomes in contrast to the normal microspore shown in figure 12.

FIGURE 14.—The first meiotic division of the diploid plant 48. Four pairs of chromosomes have already reached the poles; one pair is in the process of passing to the poles and has the appearance of the anaphase chromosomes, while the sixth pair is in the metaphase condition.

FIGURE 15.—Late anaphase of the first meiotic division of plant 48 illustrating a typical chromatid bridge.

FIGURE 16.—A normal late anaphase of the second meiotic division of the diploid plant 48.

FIGURE 17.—A normal metaphase of the first meiotic division of the triploid plant 15 with seven rod bivalents and two ring bivalents.

FIGURE 18.—First anaphase of the same triploid as in figure 17, showing an irregular distribution of the chromosomes with seven at one pole and eleven at the other. A fragment can also be seen at one pole.

FIGURE 19.—Anaphase of the microspore division of plant 15. This is from a microspore with nine chromosomes.

observed in over a dozen plants; in other plants no fragments were found although a large number of cells were carefully studied. The observations which were made can be summarized as follows, the numbers representing the individual plants which were observed:

	PLANTS WITH FRAGMENTS	PLANTS IN WHICH NO FRAGMENT WAS OBSERVED
Diploids:		
Meiosis	68	44, 70
Microspore division	68	36, 40, 42, 69, 72
Tetraploids (*canal. hirsut.*):		
Meiosis		20, 37, 73, 80
Microspore division	27, 29, 35, 45, 53, 54, 63	43, 55, 57, 58, 62, 64
Tetraploids (three species):		
Meiosis		21
Microspore division	19, 21, 38, 50, 56, 66, 67	25, 33, 41, 60, 61, 65

It was especially noted that in some plants some cells contained a fragment (occasionally two) but that other cells did not.

Summing up the diploids, it can be said that they appear, with the exception of a few genes, to be typical plants of *T. paludosa* and that a small percent of various abnormalities appears during microsporogenesis. Diploids which show a few traits of *T. canaliculata* and *T. hirsutiflora*, such as plants 3 and 36, might have had an origin as pictured in figure 20a.

Triploids

Three of the 93 plants had a haploid chromosome number of nine. Plant 15 resembled the diploid species in distribution of stomata, stomatal shape, stoutness of stem, and size of bud, was like *T. canaliculata* in leaf shape and resembled *T. hirsutiflora* in pedicel color and pubescence. Plant 15 had six nodes which is characteristic of both *T. paludosa* and of intermediates between the two tetraploid species, and it had fewer hairs on the sepals than do typical plants of *T. hirsutiflora*. Further, it had a straight stem as do plants of both tetraploid species. While this plant was unusually fertile for a triploid, since about 13.5 percent of its pollen grains appeared to be viable, it was unmistakably a triploid. Figure 17 illustrates a perfectly normal metaphase of the first meiotic division showing two ring bivalents and seven rod bivalents. An anaphase is shown in figure 18 with apparently seven chromosomes at one pole and eleven at the other along with a fragment, while a normal microspore anaphase is seen in figure 19.

Plants 24 and 83 also had a haploid number of nine chromosomes. They differed from each other and also from plant 15 in their morphological characters, but all three show definite traits of *T. paludosa* and of one or both tetraploids. Plant 24 resembled the diploid species in distribution of stomata, shape of leaf, stoutness of stem and hairiness of pedicels. In stomatal shape, it was like *T. hirsutiflora* and the number of nodes was 7, which is characteristic of *T. canaliculata*. The buds of this plant were intermediate between the small size of buds of *T. paludosa* and the larger ones of the tetraploids. The purple on the pedicels and the pubescence of the sepals were intermediate between the extreme condition of *T. hirsutiflora* and the absence of these features as in the other species; the stem was straight as in both tetraploids. The third triploid, plant 83, resembled *T.*

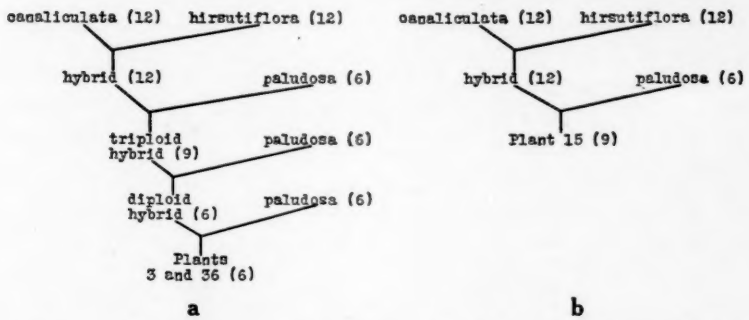

FIGURE 20a.—A diagram showing the possible origin of plants 3 and 36. These were diploids but showed some influence of both tetraploid species, and probably arose by a cross of a tetraploid hybrid with *T. paludosa* to produce a triploid which was then crossed back to *T. paludosa;* a diploid segregate of this cross probably crossed with the normal diploid species to produce plants 3 and 36.

FIGURE 20b.—A similar diagram showing the possible origin of the triploid plant 15. This probably arose directly from a cross between the diploid and tetraploid plants.

hirsutiflora only in shape of leaf, but had an intermediate number of nodes and an intermediate quantity of purple pigment; the stem was straight as in both tetraploids, but in the other characters, that is, distribution of stomata, shape of stomata, stoutness of the stem, size of bud, and pubescence of pedicels and sepals, this plant was identical with *T. paludosa*. Plant 24 had less than four percent good pollen while plant 83 was only 0.1 percent fertile. Cells in the metaphase of the first meiotic mitosis of plant 24 showed an occasional ring and rod trivalent and numerous univalents. Since plant 15 shows traits of all three species, and since it is a triploid, one might suppose that it had an origin somewhat as diagrammed in figure 20b. This assumes that the two tetraploid species had intercrossed

at some time in the past and had produced a tetraploid with characters of both species; if this hybrid was then crossed with a diploid *T. paludosa*, a triploid would have been produced which might have resembled plant 15.

Tetraploids of all three species

Twenty-one plants were tetraploids but showed evidences of contamination with the diploid species to a greater or lesser extent. These plants were relatively easy to score for stomatal distribution, leaf shape, shape of stomata, and stoutness of stem. With respect to straightness of stem and size of buds, the two tetraploids are essentially alike and both are very different from *T. paludosa;* therefore, these characters were scored as merely tetraploid-like or *paludosa*-like for these 21 plants and were disregarded when describing the tetraploids which did not show contamination with *T. paludosa*. With respect to purple color on the pedicels and pubescence of pedicels and sepals, plants of *T. canaliculata* and of *T. paludosa* are essentially alike, so these characters were disregarded for this analysis. Since many plants of *T. paludosa* have node numbers intermediate between those of the two tetraploids, this character was also disregarded.

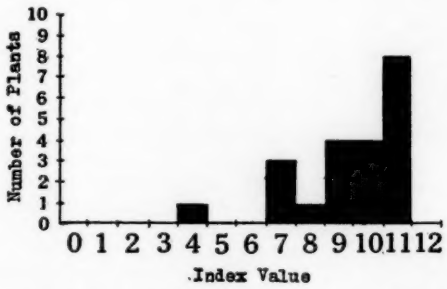

FIGURE 21.—Distribution of the tetraploids which had traits of all three species, according to their index values, the determination of which is discussed in the text.

The method of index values can be readily used for comparing these plants. In applying this method, any character resembling either of the tetraploid species was given the value 2; characters resembling *T. paludosa* were scored 0 and intermediates were assigned the value 1. On this basis a tetraploid with traits of *T. canaliculata* and *T. hirsutiflora* only would have an index value of 12 while a plant like *T. paludosa* in every respect would have an index value of 0. Figure 21 shows graphically that eight plants had values of 11, four were 10, four had an index of 9, one had an index of 8, three were 7, and that one plant was nearer the diploid and had an index

value of 4. This distribution shows clearly that this group of 21 tetraploids is much closer to the two tetraploid species morphologically than it is to the diploid. Of the 126 characters examined on the 21 plants, 27 resembled *T. canaliculata* and 32 resembled *T. hirsutiflora;* six characters were intermediate between these two tetraploids and 21 were like both (in straightness of stem and size of bud the two tetraploid species are alike). In contrast to these 86 tetraploid characters, only 15 characters on all 21 plants resembled the diploid species, and 25 characters could be interpreted as somewhat intermediate between *T. paludosa* and a tetraploid, and therefore as showing some influence of the former species.

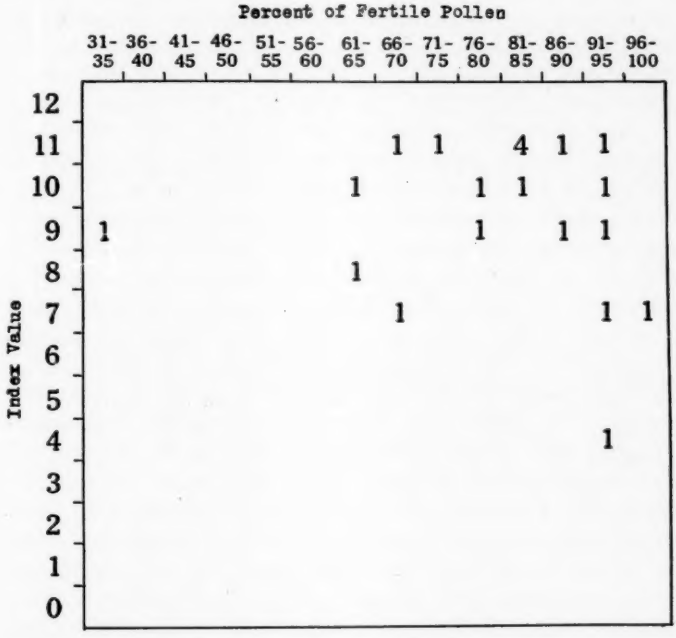

FIGURE 22.—Scatter diagram of 21 tetraploids which show traits of all three species. Percent of fertile pollen is plotted on the X axis and index value on the Y axis. There is more evidence of a curvilinear correlation here than is shown in figure 4.

In fertility of pollen, these tetraploids ranged from 33.8 to 95.5 percent. When a scatter diagram is made (figure 22) with percent of fertile pollen as the X axis and index value as the Y axis, their correlation may be compared with that of similar features of the population of non-*paludosa* tetraploids. In figure 4, no correlation is indicated for the *T. canaliculata* ×

T. hirsutiflora hybrids, but figure 22 indicates correlation in the case of tetraploids which show traits of *T. paludosa*. This correlation is curvilinear, showing that plants with either high or low index values are generally more highly fertile than those of an intermediate value. For these *T. paludosa*-tetraploid hybrids, $\eta_{xy}=0.4231$ and $\eta_{yx}=0.6152$. This difference between tetraploids involving *T. canaliculata* and *T. hirsutiflora* only and those which show an influence of *T. paludosa* is interesting. It may be due to the fact that chromosomal segments of *T. paludosa* when present in plants which are largely made up of chromosome material of *T. canaliculata* and *T. hirsutiflora* form a poorly balanced combination which exhibits more sterility than plants made up of the last two species only. On the other hand, it may mean that hybridization between *T. canaliculata* and *T. hirsutiflora* is much older than that which includes *T. paludosa*. In the case of Iris hybrids, the author (RILEY 1939 and unpublished data) found that plants of *I. fulva* and *I. hexagona* var. *giganticaerulea* were more highly fertile than hybrids of very recent origin (ten years or less). These hybrids had had little time to intercross and to backcross and differences in sterility had not been eliminated. Hybridization between *Tradescantia canaliculata* and *T. hirsutiflora* is much older and relatively common. These species are ruderals (ANDERSON 1939) and are very much at home in the gravelly soil of a railroad embankment where they frequently come close together and intercross; wherever the author has seen these species in southeastern Louisiana, they have been in such areas and have shown many evidences of hybridization. Even though the hybrids which were first produced may have been decidedly more sterile than the original species, there was enough time for frequent intercrossing; this resulted in plants with a wide range of index values and eliminated any original correlation between index values and sterility that may have existed. The tetraploids that show characters of the diploid, *T. paludosa*, are less widespread in Louisiana and are probably of more recent origin. There has been less intermingling and backcrossing and, as a result, any original correlation which may have existed between index value and sterility has not been so completely eliminated and the intermediates tend to be more sterile than plants whose index values are higher or lower.

DISCUSSION AND CONCLUSIONS

Interesting features of this population are the chromosomal and ecological barriers to hybridization and the double introgression of *T. canaliculata* into *T. hirsutiflora* and of *T. paludosa* into the tetraploids. *Tradescantia paludosa* is very abundant in southeastern Louisiana and hundreds of plants were found in the woods near the track at Michaud. The tetraploids are more frequent in the northern part of the state and in adjoining

states and when found in southeastern Louisiana are usually limited to railroad rights-of-way and are invariably hybridizing.

In establishing the particular population reported in this paper, the sequence of events may have been somewhat as follows: a few seeds of both *T. canaliculata* and *T. hirsutiflora* or more probably of hybrids between them were carried to this embankment from a distance and became established in the region near the track; these then intercrossed and formed a large population of tetraploids which exhibited an introgression of *T. canaliculata* into *T. hirsutiflora*. Seeds of *T. paludosa* were transported to the same region from the adjacent woods and became established on the lower part of the slope and in the land below the slope. A superficial examination of the unrecorded plants of the area disclosed many plants of *T. paludosa* in the flat land at the foot of the slope and especially in and along both sides of the path; these plants were not examined in detail and serve merely to supplement the original 93 and to afford a somewhat more inclusive picture of the area as a whole. When these diploids became established on the embankment in close proximity to the tetraploids, they crossed with the tetraploids forming a few triploids which then crossed back to both the diploids and tetraploids. This backcrossing accounts for the diploids with a few tetraploid characters and for the tetraploids which have some indication of *T. paludosa* in their ancestry.

A similar instance of intensive hybridization between *T. canaliculata* and *T. hirsutiflora* was observed along the tracks of another railroad within a few miles of the crossing at Michaud, but *T. paludosa* was not involved in that population. A suggestion that hybridization in southeastern Louisiana involving these three species has occurred frequently is indicated by occasional abnormalities in meiotic behavior of the chromosomes in the tetraploids and in plants of *T. paludosa*, for many plants of this diploid species show a small percentage of abnormalities such as chromatid bridges, non-disjunction and fragments. Such hybridization, however, was probably a remote one for immediate instances, as reported in this paper, have been observed in only a few rather restricted areas. The writer's studies of both Iris and Tradescantia have indicated that natural hybridization appears to be fairly common in southeastern Louisiana. Whether hybridization is more common there than elsewhere cannot be stated without a more thorough study of that and other localities, but the recent geological formation of that part of the continent would suggest that, while hybridization might not be more common then than it had formerly been in other localities, it is and has been more common recently and its evidences have not been obliterated by time. The formation of a new area would open the possibility of invasion and colonization by species from elsewhere. Such species could intercross and produce many more

hybrids than are found in land of greater age. Growing deltas may very probably be regions where active hybridization is much more frequent than it is in most areas of the world.

The writer wishes to thank PROFESSOR EDGAR ANDERSON and PROFESSOR KARL SAX for criticizing the manuscript and for helpful suggestions.

SUMMARY

1. A population of Tradescantia growing on a railroad right-of-way at Michaud crossing just outside New Orleans, Louisiana, was studied morphologically and cytologically. Ninety-three plants were examined for characters of *T. canaliculata* Raf., *T. hirsutiflora* Bush and *T. paludosa* Anders. and Woods., for fertility of pollen and for number of chromosomes.

2. Fifty-four plants were tetraploids and had some characters which resembled *T. canaliculata*, some which resembled *T. hirsutiflora* and some which were intermediate between these two species. Each of eight characters was recorded as resembling either species or as intermediate. There were 53 different combinations in these 54 plants. All the plants were tetraploids and the chromosome behavior was regular. Pollen fertility varied from 47.9 to 97.1 percent and there was no correlation between pollen fertility and morphological characters

3. Fifteen plants were diploids. Four were apparently pure *T. paludosa* but the other eleven showed a slight influence of *T. hirsutiflora* or of *T. canaliculata*. Fertility of pollen ranged from 66.7 to 98.9 percent with most plants highly fertile. Meiotic divisions showed some interlocked chromosomes, lagging, chromatid bridges, and non-disjunction; in some plants a small percent of tetraploid microspores was observed.

4. Three triploids were found in the population. All three showed morphological characters of both diploid and tetraploid species and all were highly sterile and exhibited characteristic irregularities in the behavior of their chromosomes.

5. Twenty-one plants were tetraploids but showed some traits of the diploid species. Those which were intermediate morphologically between *T. paludosa* and the tetraploids tended to be less highly fertile than those that more nearly resembled either the diploid or the tetraploids.

6. A mode of origin of this population is suggested.

7. The meiotic abnormalities in the diploids suggest that while these plants are morphologically inseparable from *T. paludosa*, they are subject to introgression from the tetraploid species.

8. It is suggested that hybridization may occur more frequently in actively growing deltas (such as that of the Mississippi River) than in older areas.

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MULTIPLE ALLELES AND SEX DETERMINATION IN HABROBRACON[1]

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INTRODUCTION

THE occurrence of biparental males in the wasp, *Habrobracon juglandis* (Ashmead) demonstrates that sex is not determined simply by the fertilized or unfertilized condition of the eggs. When females having any recessive character are mated to unrelated males with the dominant allele, the male offspring exhibit the recessive trait, the female offspring the dominant. If the parents are related, some males appear which, unlike their normal haploid brothers, show the dominant trait. These biparental males have 20 chromosomes like their sisters, in contrast to the 10 of their haploid brothers.

The first satisfactory explanation for the determination of sex in this wasp was suggested by WHITING (1933a), who proposed that femaleness is caused by heterozygosity for an allelic pair of sex factors or chromosomes, X and Y, along with two sets of autosomes. Thus the chromosomes of a female are 2A+X+Y. Haploid males are A+X or A+Y Diploid males are 2A+XX or 2A+YY Two lines of evidence support this explanation.

First, the genitalia of bilateral mosaics, produced by virgin mothers, frequently show feminization if the line of demarcation between the two types of tissue passes near the genitalia. This feminization is assumed to be caused by a substance produced by the Y factor which diffuses into the X tissue. These mosaic males result from the simultaneous cleavage of two haploid nuclei, genetically different.

Second, the locus for fused (*fu*) antennae was found to be linked with sex-determining factors (WHITING 1933b). A certain fused male, designated *fu*.X, was crossed with two of his daughters. This mating yielded 14 type daughters and one fused daughter in contrast to the 1:1 ratio which might have been expected. To show the linkage, the mating may be expressed as follows: *Fu*.Y/*fu* X ♀ ♀ by *fu*.X ♂ ♂, producing as offspring 14 type females, *Fu*.Y/*fu*.X, and one fused female, a crossover, *fu*.Y/*fu*.X. Subsequent crosses gave about ten percent crossing over between *fu* and the sex factors. Experiments reported by BOSTIAN (1935a) were made so that haploid and diploid males could be distinguished by their eye color. Orange-eyed females heterozygous for fused were crossed with closely re-

[1] The author is indebted to PROFESSOR P. W. WHITING for supplying stocks and for suggestions in the preparation of the manuscript.

lated black-eyed fused males. Results are recorded in table 1. In Group 1 the percentage of *fu* among females is 82.7, while in Group 2 the percentage is 10.9. Crossovers are more frequent when represented by type females, as in Group 1, than when represented by *fu* females, as in Group 2, because of viability differences. Attention is called to the greater occurrence of type diploid males when fused females are in excess, as in Group 1, and the reverse condition as in Group 2.

This theory of sex determination had immediately one important difficulty facing it, the need for explaining the relative infrequency with which biparental males appear. If females are XY and haploid males either X or Y, half of all fertilized eggs should be either XX or YY, and hence produce diploid males. The fact is, however, that when unrelated wasps are crossed, no diploid males at all appear, and even in fraternities from related parents they may comprise less than one percent of the biparental offspring or they may range as high as 25 percent or more from crosses of certain stocks (BOSTIAN 1934).

TABLE 1

Sex linkage of fu as shown by diploid males and females.

FRATERNITIES		PARENTS		OFFSPRING					
				♀♀ BLACK EYES		DIPLOID ♂♂ BLACK EYES		HAPLOID ♂♂ ORANGE EYES	
Groups	No.	♀♀	♂♂	+	*fu*	+	*fu*	+	*fu*
1	13	*o/o; Fu*.X/*fu*.Y	*O fu*.X	96	506	92	16	220	197
2	40	*o/o; Fu*.X/*fu*.Y	*O fu*.Y	1575	193	41	230	577	510

This difficulty has been explained in part by differential mortality. The homeosyngamic male-determining combinations (XX and YY) are less viable than the heterosyngamic female-determining (XY) ones.

This reduced viability, however, cannot explain the total absence of biparental sons of outcrossed females, for in such fraternities egg hatchability and fecundity are high and the ratio of females to haploid males is practically doubled. It had been observed by several investigators that fewer offspring are obtained from mated females than from unmated, and from close crosses than from outcrosses. Counting of eggs with daily observations on their development (BOSTIAN 1935a) established a much reduced hatchability of eggs and increased mortality of larvae and pupae for matings which produced diploid males, as compared with matings which did not include any diploid males among the offspring.

Thus WHITING (1935) was led to suggest a theory of differential maturation of the egg. "The sex chromosomes of the sperm nucleus must deter-

mine that the innermost chromosome set shall contain the opposite type of sex chromosome." According to this theory all zygotes from outcrosses are thus female-producing, heterosyngamic, XY. When parents are related, more or less homeosyngamy occurs. Thus for any particular cross the production of diploid males depends first on the relative frequency of homeosyngamic combinations and second on the viability of these zygotes.

Subsequently SNELL (1935) suggested that selective fertilization was entirely unnecessary to explain the published data of WHITING, BOSTIAN, and others, if femaleness results from the heterozygosity of one or more of a number of sex factors, some of which are located on different chromosomes. Granting the operation of five or more such pairs of factors, the chance of unrelated parents giving diploid males in the F_1 becomes very slight.

According to SNELL, the locus fused is linked with one pair of these factors, and it may be expected that further linkage tests would reveal other sex factors on different chromosomes. Furthermore it would be possible for parents to be alike for the factors linked with fused, in which case sex determination would depend on other factors, and fused would not be sex-linked. The simplest kind of such a cross would be: (*Fu*.X)(*fu*.X) WZ ♀ ♀ by (*fu*.X) W ♂ ♂ ,and the resulting zygotes would yield type and fused females in a 1:1 ratio, and type and fused diploid males in a 1:1 ratio. Sex linkage of fused might also be masked in crosses of the following types: (*Fu*.X/*fu*.X)WZ ♀ ♀ by (*fu*.Y)W ♂ ♂ and (*Fu*.X/*fu*.Y)ZZ ♀ ♀ by (*fu*.X)W ♂ ♂. Here all the zygotes would be female-producing because heterozygous, XY or WZ, and of correspondingly good viability.

Before many crosses of *Fu/fu* to *fu* had been made, fraternities appeared where there was no apparent linkage of fused and XY. In these fraternities type and fused females were approximately equal in number, and, very significantly, there were no diploid males at all.

Two interpretations were suggested. WHITING (1935) proposed translocation with a break occurring between the loci of fused and sex. According to SNELL'S hypothesis, sex was being determined by factors other than those linked with fused. More recently, on the basis of data presented by DORDICK (1937) and others (unpublished), WHITING has abandoned the hypothesis of differential maturation since "fused, shown to be sex-linked in close-crossed females, appears as if non-sex-linked if similar females are outcrossed." (Quoted from a letter by permission.) The subject up to this point has been briefly reviewed and discussed by BRIDGES (1939, pp. 54–58).

NEW DATA

In this paper data are reported which throw much doubt on the theory of multiple sex factors and indicate strongly that only one series of alleles

is concerned with sex determination. In table 2 are shown three groups of fraternities from orange-eyed females heterozygous for fused crossed with black-eyed fused males. Diploid males may therefore be separated by eye color from their haploid azygous brothers. More than half of these fraternities show independent segregation of fused and sex and such fraternities tend to be larger than those with linkage, indicating high viability of zygotes. They are therefore of the "outcross" type.

TABLE 2

*Sample matings showing absence of diploid males when fused segregates independently of sex factors.**

		OFFSPRING					
		FEMALES		DIPLOID MALES		AZYGOUS MALES	
Group	Fraternities	type	*fu*	type	*fu*	type	*fu*
1	467.3	2	12	8	3	4	5
	467.4	12	13			4	2
	467.5	1	12	11	2	4	8
2	468.8	28	22			7	4
	468.9	16	21			7	7
	468.10	11	12			9	8
3	473.1	8	51	18	10	17	19
	473.6	65	74			25	20
	473.8	46	47			24	22
	475.1	59	61			11	10
	475.6	14	70	42	9	29	19
	477.3	10	43	30	3	13	11

* Orange females heterozygous for fused (*o/o; Fu/fu*) were in all cases mated to black-eyed fused males (*O fu*). In Group 1, as also in Group 2, the females were related but not sisters and were mated to the same male. In Group 3, the females were full sisters, each mated to a different male.

On the basis of SNELL's hypothesis it appeared that it ought to be possible to develop a strain where the *fu* locus would be permanently free of linkage with the sex factors. It was also desirable to obtain more extensive data to confirm the preliminary conclusion that diploid males are never produced when *fu* fails to be sex-linked. Accordingly, for two years parents for crosses were selected from fraternities without apparent sex linkage of fused. This selection was carried through about 20 generations, and during the last 11 all crosses involved sibs. During these tests no exceptions were found to the rule that sex linkage of *fu* and the occurrence of diploid males is inseparable. Without one, the other does not occur. Nor was it possible to develop a strain breeding pure for independence of *fu* and sex.

This selection toward a strain permanently free of sex linkage was be-

gun and maintained in the following way. Mating 499.7 involved a brother-sister cross, *o/o; Fu/fu* female to *O fu* male, and the offspring were 64 type ♀ ♀ (*O/o; Fu/fu*), 63 *fu* ♀ ♀ (*O/o; fu/fu*), no diploid males, and 66 orange-eyed azygous males (*o Fu* and *o fu*).

Seven of the F_1 type females, *O/o; Fu/fu*, were mated singly with *o fu* brothers, and all matings gave independent segregation. Among the offspring, diploid males could not be distinguished in appearance from azygous males.

TABLE 3

Selection for strain showing independent segregation of fused and sex factors.

GENERATION	NUMBER OF MATINGS	MATINGS SHOWING INDEPENDENT SEGREGATION	MATINGS SHOWING SEX-LINKAGE OF *fu*
1	7	7	0
2	13	11	2
3	6	5	1
4	13	9	4
5	8	5	3
6	14	9	5
7	15	8	7
8	10	7	3
9	12	10	2
10	33	26	7
11	13	10	3

Continuing the selection, two *o/o; Fu/fu* females from one of the seven matings just mentioned were crossed to an *O fu* brother. Both crosses gave independent segregation and no diploid males.

In this way selection was continued for a number of generations. Alternate generations involved as parents orange-eyed females and black-eyed males and thus the male offspring could be separated into diploid and azygous classes. In every generation the female parents were heterozygous for *fu*, and the male parents were *fu*, so that a measure of sex-linkage was always present.

In table 3 is presented a summary of this selection, as carried out from the time brother-sister matings exclusively were made. Diploid males were not separable from azygous ones in the odd-numbered generations.

DISCUSSION

The failure to develop a strain free of linkage of *fu* and the sex factors seems to be thoroughly established, and along with the consistent absence of diploid males when *fu* is indepedent of sex, necessitates a hypothesis different from the multiple factor scheme of SNELL.

As pointed out earlier, SNELL'S hypothesis would allow the controlling factors to be a pair independent of *fu*, but does not permit any explanation for the failure of diploid males to appear after inbreeding. Nor does it

account for the seeming impossibility of developing a strain permanently free of sex linkage of *fu*.

The conclusion seems warranted that any theory of sex determination must recognize the fundamental significance of the relation of sex-linkage of *fu* and the production of diploid males. This condition and all other pertinent data as well are satisfied by WHITING's suggestion (1939 and personal correspondence) that multiple alleles are involved, x^a, x^b, x^c, x^d, etc., instead of a single pair of factors as XY. Femaleness may be considered as due to heterozygosity for any two alleles, and diploid maleness to homozygosity for any one.

When this hypothesis is applied to the experiment reported above, the seven matings of generation one (see table 3), can be indicated in the following way:

$x^a.Fu/x^b.fu$ ♀ ♀ by $x^c.fu$ ♂ ♂

Haploid offspring: non-crossovers: $x^a.Fu$, type male and $x^b.fu$, fused male
crossovers: $x^b.Fu$, type male and $x^a.fu$, fused male

Diploid offspring: non-crossovers: $x^a.Fu/x^c.fu$, type female
$x^b.fu/x^c.fu$, fused female
crossovers: $x^a.fu/x^c.fu$, fused female
$x^b.Fu/x^c.fu$, type female

Here all diploids are heterozygous for the alleles, being x^a/x^c or x^b/x^c, and hence all are females.

In the second generation, eleven matings showed segregation, and two showed linkage. Here the most likely crosses of type females to their fused brothers would be $x^a.Fu/x^c.fu$ by $x^b.fu$, and their offspring would not include diploid males. The matings producing diploid males and showing sex-linkage were of two possible kinds: $x^a.Fu/x^c.fu$ by $x^a.fu$ or $x^b.Fu/x^c.fu$ by $x^b.fu$. In the first case the female is a non-crossover and the male a crossover, and in the second the female is a crossover and the male a non-crossover.

In the third generation, five of the six matings resulted in independent segregation, as would be expected from crossing the non-crossover females, $x^a.Fu/x^b.fu$, to their non-crossover brothers, $x^c.fu$. The non-fused daughters would be approximately 90 percent $x^a.Fu/x^c.fu$ and 10 percent $x^b.Fu/x^c.fu$, and the *fu* sons would be $x^b.fu$ and $x^a.fu$ in the same ratio.

In the fourth generation, nine matings gave independent segregation. They were probably the $x^a.Fu/x^c.fu$ females by the $x^b.fu$ males. The four matings giving linkage could have been $x^b.Fu/x^c.fu$ females by $x^b.fu$ males or $x^a.Fu/x^c.fu$ females by $x^a.fu$ males.

In the next seven generations fraternities of both types occurred in each series of matings, with those showing free segregation always in the majority. With sex determination depending on multiple alleles linked with *fu*, the impossibility of developing a strain where all crosses give independent segregation is apparent. By selecting parents from a fraternity which gave

segregation three different alleles become involved, and all crosses will show segregation except for the crossovers which occur.

Further critical tests of this scheme of multiple alleles are possible. Crosses of fathers to daughters, and mothers to sons, should yield diploid males even though the original parents were entirely unrelated. Unpublished data of PROFESSOR P. W. WHITING show this to be true.

That quite a large number of these alleles may exist is suggested by the consistent failure of crosses of unrelated stocks to produce diploid males. Inbreeding following such outcrosses has always resulted in diploid males, however, with increasing frequency of occurrence with more inbreeding. In fact, when very closely related stocks are crossed, it is usually true that one hundred percent of the fraternities have diploid males. The failure of the diploid males to equal the females is due to greatly reduced viability of zygotes destined to be diploid males.

This scheme of multiple alleles with heterozygosity producing femaleness and homozygosity resulting in diploid maleness is somewhat analogous to the inheritance of self sterility in Nicotiana (EAST and YARNELL 1932). In the latter case evidence for as many as fifteen alleles has been found.

SUMMARY

Failure to establish a strain free of linkage of *fu* and the sex factors and the consistent lack of any diploid males when linkage is not present make very improbable SNELL'S hypothesis of multiple factors for sex determination in Habrobracon.

A scheme of multiple alleles, as recently suggested by WHITING, (1939) with femaleness depending on heterozygosity and diploid maleness on homozygosity, fits the data and evidence known at the present time.

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INHERITED MACROCYTIC ANEMIAS IN THE HOUSE MOUSE

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INTRODUCTION

DOMINANT spotting in mice is lethal when homozygous. The homozygote is anemic and regularly dies within a week or so after birth (DETLEFSEN 1923; DE ABERLE 1925). The haematology of the anemic mice has been worked out in detail by DE ABERLE (1927), and it has been shown by GOWEN and GAY (1932) that the anemics can be kept alive and made to grow up by intraperitoneal blood injections. The surviving anemics have a completely white coat and black eyes. This completely lethal type of anemia will be referred to in this paper as W^1.

Recently, LITTLE and CLOUDMAN (1937) described a new allele of dominant spotting, which will be referred to as W^2. This new form occurred as a spontaneous mutation in a black silver strain of mice and, like W^1, produces in the heterozygote a few white spots mostly on the forehead and on the belly. This variegation is usually far less extensive than that produced by W^1, although there is some overlapping between the two types of heterozygotes. Sometimes the spotting is confined to a very few white hairs, and occasionally it is absent altogether. However, such heterozygotes ($+W^2$) can always easily be distinguished from homozygous normal mice by the fact that the coat color is somewhat diluted, and tails and feet tend to be lighter than in normal animals. The most important difference between W^1 and W^2 is that the W^2W^2 homozygotes are viable. They usually grow up to be fairly healthy black-eyed whites; some of them develop a little pigment in their ears, often asymmetrically, and a fair proportion reaches sexual maturity.

The existence of two different alleles of W afforded an opportunity to study comparatively the three anemias W^1W^1, W^1W^2 and W^2W^2, and to gain more knowledge about the mechanism which underlies their development. The present paper deals with the haematology of W^1W^2 and W^2W^2 mice and their normal sibs, and with a few physiological experiments to test the behavior of W^2W^2 mice under experimentally changed conditions. The material thus collected is of a kind which cannot be legitimately averaged, and the use of diagrammatic representation is similarly inadvisable, as this would result in the wasting of most of the information obtained. The data are therefore given in full. Histological examination of the bone marrow and the inner organs has not yet been carried out. The author

hopes to present such data together with some biochemical tests of the blood in a later paper.

THE GENERAL DEVELOPMENT OF THE THREE ANEMIAS

Through the generosity of DRS. LITTLE and CLOUDMAN, I obtained a number of W^2w mice. A male was outcrossed to females of the black agouti Pure Line Strong CBA, and the report to be presented here, so far as it is concerned with W^2 mice, is based entirely on F_2 and F_3 animals obtained from this outcross. The W^2W^2 homozygotes proved to be considerably more vigorous than the animals described by LITTLE and CLOUDMAN, and so far, most of them have reached adult life. Unfortunately, this difference between the original stock and the animals extracted from the outcross was only realized after the foundation stock had been discarded. So it remains uncertain why animals from the outcross did better than those from the original stock. A number of W^1w heterozygotes were obtained from a fancier.

The birth weight

The early weights (1–18 hours after birth) of a number of litters are given in table 1. In confirmation of DE ABERLE'S results, it was found that W^1W^1 young are considerably lighter at birth than their normal sibs. W^2W^2 young differ very little in weight from their normal litter mates at birth and W^1W^2 young have a birth weight about intermediate between the two pure types. W^1W^1 young at birth are so pale that they cannot possibly be mistaken for normal. W^1W^2 young are decidedly paler than their normal sibs at birth and can be separated from them with fair accuracy. The classification at birth of W^2W^2 young requires some practice.

TABLE 1

Birth weights of black-eyed whites (italics) and normal sibs.

LITTER												
						W^1W^1						
A	1.88	1.87	1.80	1.80	1.77	1.64	*1.50*	*1.33*				
B	1.69	1.68	1.57	1.53	1.52	1.40	*1.32*					
C	1.79	1.71	1.70	1.61	1.54	*1.35*						
						W^1W^2						
A	1.66	1.66	1.60	1.56	1.50	*1.50*	1.47	*1.44*	*1.41*	1.41	*1.34*	*1.32*
B	1.60	1.54	1.52	1.52	1.51	*1.47*	*1.45*	1.40	*1.39*	*1.36*	1.33	
C	1.55	1.51	1.46	1.42	*1.39*	*1.24*						
						W^2W^2						
A	1.51	*1.50*	1.43	1.42	*1.41*	1.39	1.37	1.35	1.31	1.12		
B	1.44	1.44	1.41	1.38	1.36	1.36	1.26	1.23	*1.23*	*1.19*		
C	1.54	1.47	1.38	*1.37*	1.36	*1.36*	1.35	1.34	1.32	1.29		
D	1.72	1.71	1.70	1.69	1.69	1.66	*1.64*	*1.56*	*1.56*	1.45		
E	*1.43*	1.42	1.40	1.37	1.37	1.35	1.34	1.26	1.25	*1.22*		
F	1.58	1.53	1.48	1.46	1.40	*1.36*						

	Averages		
	Normals (N)	Black-eyed whites (B)	B/N
W^1W^1	1.68	1.38	.82
W^1W^2	1.51	1.39	.92
W^2W^2	1.42	1.40	.99

Growth in weight

As shown by DE ABERLE and confirmed by a few new observations, W^1W^1 young are unable to put on weight. They eventually get into a state of extreme emaciation, when they either die spontaneously or are eaten

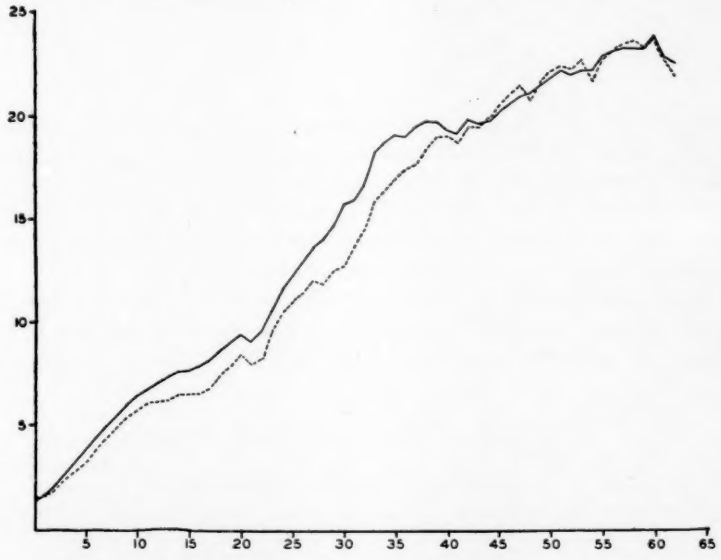

FIGURE 1.—Individual growth curve of a W^2W^2 anemic male (broken line) and compound growth curve of four normal brothers.

by their mothers. The growth curves of W^2W^2 young present a completely different picture. The young usually gain weight fairly normally. Then they may fall back and subsequently be unable to recover the ground lost. In most cases (figure 1), the black-eyed whites develop slightly sub-normally in the first week or two. The animal in figure 1 managed to attain a perfectly normal weight later, and for some time was actually the heaviest animal in the litter. The black-eyed whites often do not deviate from their normal litter mates in a very spectacular way. On the other hand, they always are somewhat less well off than the normals. This slight disadvantage is not yet present at birth. It develops during the first few days of

life, as shown in table 2, and later in life the early disadvantage is either maintained or outgrown. It seems that males have a slightly better chance to reach normal weight eventually than females, and similarly males have a better chance to become sexually mature than females.

TABLE 2

Average weights of W^2W^2 anemics and their normal sibs.

AGE IN DAYS	NORMALS (N)	BLACK-EYED WHITES (B)	B/N
0	1.42 (44)	1.40 (12)	.99
4	3.22 (43)	2.91 (12)	.90
8	5.39 (28)	4.79 (7)	.89
12	7.02 (23)	6.16 (7)	.88

There does not appear to be a significant difference between heterozygotes (W^1w and W^2w) and homozygous normals (ww), as far as their general development is concerned. The data for normals therefore include both heterozygotes and homozygous normal animals in most litters. Similarly, there seem to be no significant differences between heterozygotes and homozygous normals as regards their blood pictures.

Whereas the general development of W^1W^1 and W^2W^2 mice is generally fairly uniform, considerable variations occur in the life histories of W^1W^2 compounds. From birth up to about the ninth day of life, the compounds

TABLE 3

Average weights of W^1W^2 compounds and their normal sibs.

AGE IN DAYS	NORMALS (N)	BLACK-EYED WHITES (B)	B/N
0	1.51 (18)	1.39 (11)	.92
4	3.18 (18)	2.66 (11)	34
8	5.59 (18)	4.25 (11)	.76
12	7.76 (18)	5.10 (11)	.66

grow steadily, but at a considerably slower rate than their normal sibs. The 11 animals listed in table 3 average 92 percent of the weight of their normal sibs shortly after birth. The same 11 animals average only 76 percent of the weight of their normal litter mates at the age of eight days. The time of about nine to ten days of age appears to be critical for some of the animals. Five out of the eleven animals developed a slight transitory jaundice which was particularly noticeable on ears, belly and tail. These animals became stationary, as shown in figure 2, and died during the third week of diarrhoea (15, 15, 17, 17 and 18 days old). As none of the normal litter mates showed any signs of diarrhoea, it appears likely that this was

a symptom of their anemia. Diarrhoea is a condition very commonly recorded in various kinds of anemia in man, notably in acute haemolytic jaundice. The other six animals went on growing in a somewhat irregular manner, some gaining fairly steadily, whereas others showed gains interrupted by losses. Such fluctuations are not found in normal mice under 30 days of age, except for a day or two at weaning time. Eventually, five of the six survivors died (25, 29, 30, 31 and 38 days old), mostly after short

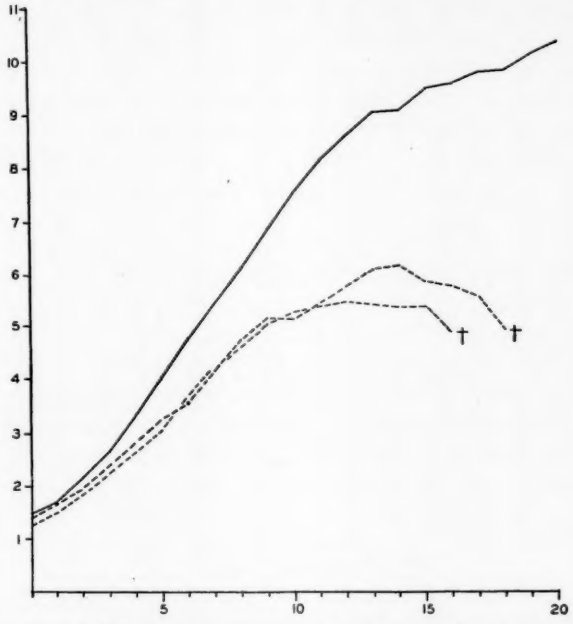

FIGURE 2.—Individual growth curves of two W^1W^2 anemics (broken lines) and compound growth curve of four normal litter mates.

periods of weight loss. One animal has grown up to be an adult, reaching a weight of about 22 grams.

THE HAEMATOLOGY OF W^2W^2 MICE

In the absence of sufficiently detailed haematological data on normal young mice in the literature, all the work has been carried out on anemics and their normal litter mates as controls. Blood in most cases was obtained by decapitation; in a few instances, animals were bled from the tails. In table 4, each animal occurs but once. The animals of 40 and 41 days are the controls in table 16.

TABLE 4

W² stock. Haemoglobin values, erythrocyte counts, color indices and reticulocyte counts.

LITTER	AGE IN DAYS	NORMALS					BLACK-EYED WHITES				
		Hb %	ERYTHROCYTES PER MM³	COLOR INDEX	RETICULOCYTES PER MM³	%	Hb %	ERYTHROCYTES PER MM³	COLOR INDEX	RETICULOCYTES PER MM³	%
14/15g	0	84	3,695,000	1.14	1,914,000	51.79	61	2,250,000	1.36	1,787,000	79.44
14/15g	0	89	4,045,000	1.10	2,229,000	55.10	59	2,005,000	1.47	1,604,000	80.00
23/24c	0+	78	3,095,000	1.26	1,954,000	63.13	52	1,895,000	1.37	1,351,000	71.29
23/24c	0+	80	3,390,000	1.18	2,016,000	59.48	52	2,000,000	1.30	1,526,000	76.30
31/32c	0+	84	3,625,000	1.16	2,215,000	61.09	57	1,980,000	1.44	1,198,000	60.50
31/32c	0+	83	3,905,000	1.06	2,265,000	58.00	55	2,090,000	1.32	1,323,000	63.30
2/3f	1	76	3,750,000	1.01	1,856,000	49.75	48	1,480,000	1.62	827,000	55.85
2/3f	1	79	4,045,000	.98	1,912,000	47.27	47	1,515,000	1.55	814,000	53.70
8/9c	3	68	3,840,000	.89	872,000	22.72	44	2,200,000	1.00	350,000	15.90
8/9c	3	70				15.54	36	1,620,000	1.11	277,000	17.06
8/9c	4	64	3,040,000	1.05	725,000	23.84	38	1,900,000	1.00	207,000	10.87
8/9c	4	58	3,240,000	.90	681,000	21.01	32				26.47
10/11e	5	54	3,880,000	.70	938,000	24.18	35	1,605,000	1.09	497,000	30.99
10/11e	5	60	3,960,000	.76	992,000	25.04					29.97
10/11e	6	64	4,540,000	.70	1,162,000	25.60	38	1,930,000	.98	587,000	30.41
10/11e	6	66	4,780,000	.69	1,255,000	26.25	40				26.09
10/11c	7	66	4,100,000	.80	1,272,000	31.03	44	2,200,000	1.00	857,000	38.94
10/11c	7	64	4,200,000	.76	1,206,000	28.72	50	2,820,000	.89	1,119,000	39.69
10/11c	8	70	5,160,000	.68	1,781,000	34.51	38	2,060,000	.92	851,000	41.31
14/15d	8	63	3,760,000	.84	907,000	24.13	42	1,690,000	1.24	507,000	30.02
14/15d	9	61	4,545,000	.67	1,144,000	25.16	40	2,145,000	.93	779,000	36.31
14/15d	9	63	4,900,000	.68	1,193,000	25.93	48	2,195,000	1.09	752,000	34.25
25/26d	10	75	5,745,000	.65	1,436,000	25.00	58	3,245,000	.89	1,081,000	33.30
25/26d	10	77	6,505,000	.59	1,685,000	25.90	48	2,550,000	.94	936,000	36.70
25/26d	11	73	5,325,000	.69	1,278,000	24.00	55	3,345,000	.82	1,054,000	31.50
25/26d	11	67	5,205,000	.64	1,218,000	23.40	50	3,080,000	.81	1,069,000	34.70
33/34a	13	55	4,790,000	.57	892,000	18.63	50	3,055,000	.82	470,000	15.40
33/34a	13	55	4,580,000	.60	695,000	15.18	40	2,740,000	.73	471,000	17.19
25/26b	15	70	6,620,000	.53	818,000	12.36	55	4,190,000	.66	727,000	17.36
2/3d	17	50	4,380,000	.57	884,000	20.19	42	3,090,000	.68	645,000	20.82
8/9b	20	60	6,640,000	.45	891,000	13.42	54	4,560,000	.59	507,000	11.11
8/9b	20	80	7,280,000	.55	1,105,000	15.18	65	4,840,000	.67	657,000	13.57
8/9b	21	76	6,200,000	.61	1,008,000	16.25	56	4,000,000	.70	300,000	7.49
8/9b	21	78	6,880,000	.57	1,289,000	18.70	64	3,900,000	.82	603,000	15.47
2/3d	22	67	7,130,000	.47	1,407,000	19.74	47	3,820,000	.62	917,000	24.01
2/3d	22	66	6,450,000	.51	1,387,000	21.51	46	3,490,000	.66	768,000	22.00
2/3d	23	66	5,770,000	.57	1,402,000	24.30	53	3,265,000	.81	750,000	23.25
2/3d	23	68	6,030,000	.56	1,520,000	25.21	40	3,025,000	.66	781,000	25.82
14/15c	33	98	9,920,000	.49	409,000	4.12	72	5,440,000	.66	499,000	9.17
14/15c	33	94	7,440,000	.63	737,000	9.91	66	5,120,000	.64	465,000	9.09
8/9d	40	90	9,250,000	.49	304,000	3.29	62	5,030,000	.62	289,000	5.75
10/11d	41	98	10,300,000	.48	472,000	4.58	82	7,770,000	.53	542,000	6.98
14/15c	47	91	9,400,000	.48	431,000	4.58	70	6,335,000	.55	324,000	5.12
x/9	75	64	7,040,000	.45	322,000	4.58	84	5,880,000	.71	534,000	9.09
x/9	78	96	8,960,000	.54	602,000	6.72					
14/15a	88	108	9,930,000	.54	649,000	6.54	74	5,990,000	.62	301,000	5.03

Haemoglobin was estimated according to Haldane's method. Two estimations were carried out wherever possible. The values are given as percentages of the human standard, a reading of 100 percent corresponding to 13.8 grams of haemoglobin in 100 cc of blood. Erythrocyte counts were made in a Zeiss-Bürker chamber in a dilution of 1/200. The cells in at least 80 and in most cases 160 small squares of an area of 1/400 mm² were counted. The counts therefore included 1/10,000 or mostly 1/5000 of the values given in table 4. A value of, say, 4,000,000 thus corresponds to at least 400, and generally 800 cells actually counted. Reticulocytes were stained supravitally on slides with brilliant cresyl blue. The counts comprise mostly between 1000 and 1200 cells or over; in a few instances, only 800–1000 cells have been counted. The color index (C.I.) has been calculated according to human standards, taking 5,000,000 erythrocytes per mm³ and a reading of 100 on the Haldane haemoglobinometer as 100 percent. The

normal mouse has blood values very different from those of man. For purposes of comparison, therefore, the values of the black-eyed whites (B) have been expressed in terms of the corresponding values of their normal (N) litter mates in Tables 5–8. The B/N values are thus a measure for the relative performance of the anemics and are independent of the absolute values which show a considerable change with age. The blood picture is usually fairly uniform within litters. There are, however, often some differences between litters, particularly when they are young, which are presumably due to differences in litter size and the care which the mothers show in nursing their young. Comparisons should therefore, so far as possible, be made between black-eyed whites and normals of the same litter. As the blood values vary strikingly with age, the averages as given in tables 5–8 are to be taken with caution; their absolute values depend very largely on the relative representation of particular age groups within each average. The use of these averages is, however, legitimate, as far as the B/N values are concerned, because these are unaffected by changes with age.

The erythrocyte counts of normal litter mates are in the neighborhood of 3,700,000 at birth. After a slight drop the figures gradually increase, and in fully adult mice (see also tables 20 and 23 below) reach about 10–11,000,000 per mm³. The corresponding counts of the black-eyed whites are consistently below those of their normal sibs, starting with 1,900,000 and gradually increasing up to about 6,500,000 per mm³, a figure as high as 7,770,000 having occurred but once in the whole material. Averages of cell counts for four age groups are given in table 5, together with the corresponding B/N values.

TABLE 5

Averages of erythrocyte counts.

AGE IN DAYS	NORMALS, ++ AND W^2+(N)	BLACK-EYED WHITES, W^2W^2(B)	B/N
0– 1	3,693,750 (8)*	1,901,875 (8)	.51
3–10	4,393,000 (15)	2,166,154 (13)	.49
11–23	5,948,571 (14)	3,600,643 (14)	.61
33–88	9,030,000 (8)	5,937,857 (7)	.66

* Number of individuals in parentheses.

Up to the age of 10 days, the anemics have about half the normal number of erythrocytes per mm³. Later, their relative performance improves to about 60 percent. The slight difference in B/N values between the last two groups is of doubtful significance. It appears that the critical time for the relative improvement of the anemics is in the neighborhood of about 10–12 days of age.

The haemoglobin values of the normals show a marked drop after birth followed by a strong increase after weaning age. A similar decline followed by an increase with age occurs in the black-eyed whites. But the black-eyed whites have distinctly lower haemoglobin values than their normal litter mates at all ages. The difference, however, is not so great as is that of the cell numbers, and actual overlaps between the values of normals and black-eyed whites within the same litter occur occasionally (see litters of 20 and 75 days of age in table 4). The following figures (table 6) are averages for the same four periods as given above for the cell counts.

The situation is essentially similar to that found in the cell counts. There is a marked improvement of the relative performance of the black-eyed whites between the second and third age groups, but evidently little further improvement later in life.

TABLE 6

Averages of haemoglobin values.

AGE IN DAYS	NORMALS, ++ AND W^2+ (N)	BLACK-EYED WHITES (B)	B/N
0– 1	81.6% (8)	53.9% (8)	.66
3–10	65.2% (16)	42.1% (15)	.65
11–23	66.5% (14)	51.2% (14)	.77
33–88	92.4% (8)	72.9% (7)	.79

It is obvious that in all age groups, the anemia of the black-eyed whites is more severe as regards the number of erythrocytes than as regards the haemoglobin. This finds its expression in the color indices which are given individually for each beast in table 4. The color index is the ratio of haemoglobin percent/erythrocyte count percent and therefore is an expression for the amount of haemoglobin contained in the individual erythrocytes; it rises and falls with that quantity. If the color index is below normal in anemia, it is called "hypochromic," and if it is above normal, it is "hyperchromic." Perusal of table 4 shows that the anemia found in W^2W^2 mice is clearly hyperchromic, that is to say, the erythrocytes are reduced more strongly than is the haemoglobin. With but one exception, which may be due to an experimental error, the C.I. is higher in the black-eyed whites than it is in their normal litter mates. Averages for the four age groups are given in table 7.

Table 7 shows that both in normals and in black-eyed whites, the color indices decrease with age, rapidly at first and slowly later on. The last column gives the C.I. of the black-eyed whites in terms of that of the normals. It is clear that the relative strength of the hyperchromia of the black-eyed whites remains practically the same in all the four age groups.

TABLE 7

Averages of color indices.

AGE IN DAYS	NORMALS (N)	BLACK-EYED WHITES (B)	B/N
0– 1	1.10	1.42	1.29
3–10	.74	.97	1.31
11–23	.56	.71	1.27
33–88	.51	.61	1.20

We have to consider next the behavior of the reticulocytes. These cells are young erythrocytes in which the basophilic remnants of the disintegrating nucleus can still be precipitated supravitally by certain stains, such as brilliant cresyl blue. The behavior of the reticulocytes is an indicator for the rate at which new cells are produced by the blood-forming organs. The reticulocyte number may either be given as a percentage of reticulocytes to red cells in general, or else the number of reticulocytes per mm^3 may be given. Both kinds of data, together with their B/N values, are given in table 8. In that table, the values for animals of 3–4 days are given separately, because this age group seems to differ strikingly both from the preceding and the succeeding age group both in absolute and relative performance.

It was shown by DE ABERLE (1927) that 15 day old mouse fetuses show reticulation in all their red cells. From the sixteenth day of gestation onwards, non-reticulated cells make their appearance, and a sudden and rapid drop occurs after birth. As shown by table 8, a low level is reached when

TABLE 8

Averages of reticulocyte percentages (upper half of table) and of reticulocyte number per mm^3 (lower half of table).

AGE IN DAYS	NORMALS (N)	BLACK-EYED WHITES (B)	B/N
0– 1	55.70% (8)	67.55% (8)	1.21
3– 4	20.78% (4)	17.58% (4)	.85
5–11	26.35% (14)	33.87% (14)	1.29
13–23	18.39% (12)	17.79% (12)	.97
33–88	5.54% (8)	7.18% (7)	1.30
0– 1	2,044,974 (8)	1,303,669 (8)	.64
3– 4	759,303 (3)	277,675 (3)	.37
5–11	1,244,754 (14)	840,673 (12)	.68
13–23	1,108,130 (12)	633,796 (12)	.57
33–88	490,820 (8)	422,281 (7)	.86

TABLE 9

Distribution of erythrocyte diameters in μ.

STOCK	AGE IN DAYS	N OR B	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5
W^2	5	N					1	1	1	7	21
	5	B		1					3	3	2
W^2	9	N	1		1	2	1	1		16	40
	9	B		2		1	2	4	5	7	13
W^2	17	N						4	10	50	69
	17	B				1	1	2	4	16	46
W^2	40	N					1	4	35	122	72
	40	B				2	1		5	33	67
W^1W^2	10	N			1		1	4	8	37	52
	10	B			1		1	5	4	4	7
	10	B				2	3	2	3	9	5

the animals are about three–four days old, and this is followed by a secondary rise which lasts from about the fifth to the 11th days. After that, the figures fall slowly, and in fully adult mice, only a few percent of the red ceils, sometimes only about one percent, still show reticulation. The black-eyed whites behave in much the same way as their normal sibs, but with some quantitative differences. The drop in the reticulocyte percentage after birth is delayed, and consequently the B/N value is higher than unity. The drop is eventually at least as low as in the normals. The secondary rise is significantly higher. This indicates that in the 5–11 days age group, the black-eyed whites are turning out erythrocytes more rapidly than normal mice. The result of this increased activity we have already seen in tables 5–7, where it is obvious that the decisive improvement of the black-eyed whites takes place at the age of about 10–12 days. It is a well-known fact, for instance in the liver treatment of pernicious anemia in man, that a rise in the reticulocyte count precedes the actual increase in erythrocyte number by several days. Later in the life of the black-eyed whites, the reticulocyte percentage is about normal or tends to be somewhat higher than in normal mice.

The situation is somewhat different when account is taken of the absolute numbers of reticulocytes per mm^3 of blood (lower part of table 8). Here it will be seen that throughout, the reticulocyte numbers of the black-eyed whites are considerably lower than those of their normal sibs. The B/N values show a minimum of only .37 at the age of 3–4 days, which

TABLE 9—*Continued*

Distribution of erythrocyte diameters in μ.

7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	TOTAL
41	53	65	42	15	3							250
14	18	42	44	37	31	31	14	7	1	1	1	250
72	48	36	20	8	1							247
30	47	74	45	15	3	1	1					250
72	32	13	1									251
58	72	40	9	1		1						251
18	1											253
77	39	23	6									253
66	47	20	11	3								250
20	48	76	57	18	7	2						250
15	33	53	53	50	18	4						250

is followed by an increase to .68. After weaning, the absolute reticulocyte figures of the black-eyed whites become very nearly normal, and actual overlapping of the values within the same litter is found frequently.

To sum up: whereas the relative frequency of reticulocytes tends to be higher than normal in most age groups in the black-eyed whites, the absolute number of reticulocytes as turned out by the blood-forming organs is always seriously reduced, and only in adult life may the output of reticulocytes become more or less normal.

The size of the erythrocytes was determined by projecting stained smears at a magnification of 1000 times and drawing the outlines of about 250 cells per smear. The diameters were then measured to an accuracy of 0.5 mm. The distributions (PRICE-JONES 1933) for four pairs of animals are given in table 9. The mean cell diameters (M.C.D.) with their standard errors and the coefficients of variation (v), together with B/N values for four pairs of animals are given in table 10.

In the normal mouse, as in man, the erythrocytes are bigger in infancy than in adult life. The transition from the megaloblastic erythropoiesis to normoblastic erythropoiesis, though it starts before birth, is not complete before the animal is about two months old. The cell size in youngsters is very variable, and a small percentage of very small microcytes appears to be physiological. Later, the cell size becomes much more uniform, as shown by the decrease in the coefficient of variation. The W^2 anemics behave in essentially a similar way, but at every stage, the erythrocytes are sig-

TABLE 10

Mean red cell diameters and coefficients of variation.

AGE IN DAYS	NORMALS (N) M.C.D. (μ)	NORMALS (N) v PERCENT	BLACK-EYED WHITES (B) M.C.D. (μ)	BLACK-EYED WHITES (B) v PERCENT	B/N
5	7.675± .0512	10.53	8.764± .0773	13.95	1.14
9	7.219± .0581	12.66	7.656± .0658	13.59	1.06
17	6.693± .0409	9.68	7.159± .0479	10.60	1.07
40	6.128± .0277	7.19	6.877± .0447	10.33	1.12

nificantly bigger (*megalocytosis*). At the same time, the cell size is more variable (*anisocytosis*). The anisocytosis is, however, not very marked.

The B/N values do not seem to vary systematically with the age of the animals. It appears that on an average the W^2 anemics have cells with a mean diameter about 1.10 times that of a normal mouse. Neglecting the thickness of the cells, the mean area of the red cells would be about $1.10^2 = 1.21$ times that of normal cells. As the cells of the anemics are presumably proportionally thicker than those of normal mice, we may estimate that the mean cell volume is about 1.33 times that of a normal.

Now it will be remembered that the mean color index of the W^2 anemics (B/N) is about 1.28. As the cell size is increased to about the same extent, the actual concentration of haemoglobin per unit *volume* of erythrocyte is about normal. In other words, the erythrocytes of W^2 anemics are in fact *orthochromatic*, and the apparent hyperchromic behavior, as reflected in the raised color index, is solely due to the fact that that index does not take account of the increased average cell size.

That the erythrocytes of the anemics are orthochromatic is also shown by stained smears. Genuinely hyperchromic cells stain more intensely than normal cells, but this was not observed in W^2W^2 animals. The staining behavior of the erythrocytes is fairly uniform, apart from the polychromatophilia (see reticulocyte-values above) and thereby differs from human pernicious anemia (ADDISON-BIERMER), where there is often a mixture of very pale and very intensely stained cells. It should be added that normoblasts are practically absent, and that not a single megaloblast has been observed. The absence of these cells is another difference from most cases of pernicious anemia in man, though cases are not uncommon where such nucleated cells are practically or entirely missing.

The orthochromatic behavior of the erythrocytes of the W^2 anemics was confirmed more directly by haematocrit investigations. 20, 40 or 60 mm^3 of blood was diluted with 5 cm^3 of saline (.93 percent NaCl solution) and spun down at 3000 revolutions for 15 minutes in a thick tube ending

in a graduated thinner tube. In this way, the volume of the packed erythrocytes can be estimated. From the same animals, erythrocyte counts were made and duplicated haemoglobin estimations were carried out. The cell counts, with the exception of those of the five days old animals, comprised 1/2500 of the figures given in table 11; the estimates are therefore more accurate than those presented in table 4, where 1/10,000 or 1/5000 were counted. In the case of the five days old animals, the counts amounted to 1/4167. The simultaneous estimation of cell number, haemoglobin and volume of packed cells allows the calculation of the color index, the mean volume of erythrocytes in μ^3 and the haemoglobin concentration per unit volume of erythrocytes, equating a reading of 100 percent of the Haldane haemoglobinometer to 13.8 grams of haemoglobin per 100 cm^3 of blood The results, together with B/N values for each pair of estimates, are given in table 11.

TABLE 11

Erythrocyte volume and corpuscular haemoglobin concentration.

AGE IN DAYS	N OR B	ERYTHROCYTES PER MM^3	Hb %	VOLUME OF PACKED CELLS %	COLOR INDEX	MEAN CELL VOLUME IN μ^3	MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION %
5	N	4,754,167	64	45.00	.673	94.653	19.63
	B	1,766,667	35	25.00	.991	141.509	19.32
	B/N	.372	.547	.556	1.473	1.495	.984
10	N	5,697,500	70	42.50	.614	74.594	22.73
	B	3,795,000	56	37.50	.738	92.227	20.61
	B/N	.666	.800	.882	1.202	1.236	.907
15	N	6,150,000	64	38.50	.520	62.602	22.94
	B	3,682,500	51	32.50	.692	88.255	21.66
	B/N	.599	.797	.844	1.331	1.410	.944
31	N	9,380,000	86	53.50	.459	57.037	22.18
	B	5,042,500	64	41.20	.635	81.639	21.45
	B/N	.538	.762	.770	1.383	1.431	.967
171	N	10,837,500	105	55.75	.484	51.442	25.99
	B	6,705,000	87	46.00	.649	68.811	26.10
	B/N	.623	.829	.825	1.341	1.338	1.004
Average	B/N	.560	.747	.775	1.346	1.382	.961

The cell counts and haemoglobin values agree well with those given in table 4. It will be noticed that the B/N values for haemoglobin differ very little from those for the volume of packed cells. The averages are .747 and .775 respectively, an agreement within 4 percent, and the biggest individual difference (.800 and .882) is only just over 10 percent. This clearly indicates that the haemoglobin concentration per unit volume of erythrocytes is practically normal in the W^2 anemics. The actual haemoglobin concentrations are given in the last column of table 11. The differences between normals and anemics are negligible, and there cannot be any doubt that the erythrocytes of the anemics are, within the accuracy of the measurements, orthochromatic.

The mean erythrocyte volume varies strikingly with the age of the animals. It is high at first, drops rapidly between the fifth and fifteenth day, and then slowly approximates adult values. Throughout, the cell volume of the anemics is substantially higher than that of the normals. The data thus agree excellently with the mean cell diameter measurements reported above. It is an interesting fact that over the whole range of values, the B/N values for the color indices are practically the same as those for the cell volumes. The averages are 1.346 and 1.382 respectively and therefore agree to within 2.7 percent. This means that in our case, the color index of the anemics as expressed in terms of that of the normals is not an indicator for the haemoglobin concentration, but an index for the relative volume of the average erythrocyte. Similarly, and this is an important point, the changes in the color index with age, which occur both in normals and anemics, are mainly the expression of a change in cell volume. But, whereas the color index decreases with age, the mean corpuscular haemoglobin concentration seems actually to increase slightly. This slight change in the opposite direction is completely obscured by the color index.

In some human anemias, notably in haemolytic (acholuric) jaundice, the shape of the erythrocytes is altered. In that condition, the cells tend to be sub-globular instead of being flat discs with shallow depressions in the middle. The data reported above enable us to answer the question whether a similar shape anomaly is present in the erythrocytes of the W^2 anemics.

From the mean cell diameters, the average surface area of the discs can be calculated (area $= d^2\pi/4$). Neglecting the depressions and thus treating the cells as short cylinders, the average thickness of the cells is given by the ratio of volume/area. The ratio diameter/thickness (D/T) is then a convenient measure for the relative thickness of the cells. As some of the diameter measurements were done at ages not exactly corresponding to those of the volume measurements, the diameters, except those for five days, were recalculated by linear interpolation, and eventually the values given in table 12 were obtained.

TABLE 12

Relative thickness of erythrocytes in normals and W^2 anemics.

AGE	NORMALS					BLACK-EYED WHITES				
	DIAMETER	AREA	VOLUME	THICKNESS	D/T	DIAMETER	AREA	VOLUME	THICKNESS	D/T
	μ	μ2	μ3	μ		μ	μ2	μ3	μ	
5	7.675	46.3	94.7	2.046	3.751	8.764	60.3	141.5	2.346	3.736
10	7.153	40.2	74.6	1.856	3.854	7.594	45.3	92.2	2.036	3.730
15	6.815	36.5	62.6	1.716	3.971	7.283	41.7	88.3	2.119	3.437
31	6.349	31.7	57.0	1.802	3.523	6.987	38.3	81.6	2.129	3.282
Average D/T values					3.775					3.546

An idea about the accuracy of agreement to be expected may be obtained by a comparison of the D/T ratios within the groups of normals and anemics at different ages. These differences are obviously accidental and due to the limits of accuracy of measurement and individual variations. The biggest difference between normals is about 12.7 percent and that between anemics 13.8 percent. The difference between the average D/T values of normals and anemics is only 6.5 percent and therefore clearly insignificant. We may conclude that, within the limits of accuracy of measurement, there is no significant difference in the relative thickness of the red blood corpuscles.

In human acholuric (haemolytic) jaundice, the resistance of the red blood corpuscles against hypotonic salt solutions is lessened; in other anomalies of the blood, it may be increased. It seemed desirable to ascertain whether the black-eyed white anemics differed in any way from normals as regards the resistance of their erythrocytes. Experiments with NaCl solutions ranging from .70 per cent to .35 percent were therefore carried out. Blood was obtained by decapitation and slightly diluted with a heparin solution in .85 percent saline to prevent clotting. One drop of blood was added to one or two cc of the saline solutions in small test tubes; the test tubes were carefully turned twice to obtain a homogeneous mixture of blood and saline, and the result was read a few hours later after sedimentation had taken place. The results are summarized in table 13, where +++ stands for complete haemolysis, and ++, +, (+) stand for decreasing intensities of incomplete haemolysis.

The table shows clearly that at no stage is there any sign of an increased fragility of the erythrocytes of the W^2 anemics. The corpuscles of very young animals seem to be more resistant to hypotonic salt solutions than those of older animals, but in each case, normals and black-eyed whites behave alike.

It should be pointed out that this negative result does not in itself ex-

TABLE 13

Fragility tests of red blood corpuscles.

AGE IN DAYS	N OR B	NaCl CONCENTRATION .70	.65	.60	.55	.50	.45	.40	.35
10	N ♀	−	−	−	−	−	+	++	++
10	B ♀	−	−	−	−	−	+	++	++
30	N ♂	−	−	−	(+)	+	++	+++	+++
30	B ♂	−	−	−	(+)	+	++	+++	+++
150	N ♂	−	−	−	+	++	+++	+++	+++
150	B ♀	−	−	−	(+)	+	++	+++	+++

clude the possibility that the anemia of the W^2W^2 animals is of a haemolytic nature. The resistance to hypotonic salt solutions may be normal, and yet the resistance to other agents may be decreased. What the experiment does show is that the mouse anemia differs from human acholuric jaundice in a symptom which is regarded as pathognomonic in the human disease.

A few platelet counts have been carried out. The results are given in table 14. The averages agree to within 10 percent. Considering the comparatively low accuracy of such counts and the fact that the platelet values even of normal individuals may vary to a considerable extent, this difference is to be regarded as trivial.

Leucocyte counts of W^2 anemics and their normal sibs are summarized in table 15.

It will be noticed that almost all the figures, normals as well as anemics, are very low indeed. Such a leucopenia seems to occur in mouse colonies fairly frequently without its reason being known. It is mentioned, for instance, by C. KLIENEBERGER (1927). In our mouse colony, the animals enjoy perfect health, and there is no indication whatever of infectious dis-

TABLE 14

Platelet counts; W^2 stock.

LITTER	AGE IN DAYS	NORMALS	BLACK-EYED WHITES
W^2 8/9b	20	610,880	551,760
W^2 8/9b	20	975,520	1,064,480
W^2 8/9b	21	613,800	368,000
W^2 8/9b	21	639,840	604,500
W^2 x/9	75	704,000	658,560
W^2 x/9	75	734,720	
Average		713,127	649,460

eases which might account for it. It is certain that the leucopenia has nothing to do with the anemia under discussion. This is evidenced by the fact that homozygous normal mice (figures in *italics*) do not differ significantly from heterozygotes. The B/N values show that anemics have on the whole lower white cell counts than their normal sibs, though there is a good deal of overlapping. The B/N values further show that the white cells are considerably less reduced than the erythrocytes. After weaning the difference seems to be negligible, though the material is far too scanty to be certain about this point.

TABLE 15

Leucocyte counts. Homozygous normal mice in italics.

AGE IN DAYS	NORMALS (N) INDIVIDUAL COUNTS			AVERAGE	BLACK-EYED WHITES (B) INDIVIDUAL COUNTS			AVERAGE	B/N
6	3001	2633	*2489*		2067	1922	1833		
	2444	*1956*	1956	2413	1367			1797	.74
9	*5700*	4422	4422		3156	3067	3022		
	4356	*3867*	3656		2222				
	3500	2556		4060				2867	.71
16	2589	*2211*	2078		1922	1622	1378		
	1778	1711		2073				1641	.79
18	2911	2556	2282		2011	1878	1867		
	2000			2437				1919	.79
29	2033	*1776*	*1756*		1756	1667	1422		
	1567	1522		1731				1615	.93
184	7437			7437	8400			8400	1.13

Differential counts were carried out in a number of cases. Sometimes, but by no means always, did the W^2 anemics show a relative lymphocytosis, the percentage of lymphocytes exceeding the usual 75 percent. In other cases, the differential count did not differ significantly from that of the normal litter mates. It appears unnecessary to give all these figures in detail. It should be pointed out, however, that in no case did immature white cells make their appearance in the circulation of W^2 anemics. The differential counts therefore seem to indicate that in some cases at least, the production of granulocytes is more seriously affected than that of lymphocytes.

To sum up; the W^2 anemics suffer from a comparatively mild form of macrocytic anemia. The haemoglobin concentration per unit volume of cells is normal. The cells are of normal shape and show a low degree of

anisocytosis. Their resistance to hypotonic salt solutions is normal. No nucleated red cells occur in the circulation. The reticulocytes are relatively increased in number in most age groups, but their absolute number per unit volume of blood is strikingly reduced, except in adult life, when their absolute number may become practically normal. Platelet counts are normal. The leucocytes are reduced in number, though to a lesser extent than the red cells. There is some indication that granulocytes are more strongly affected than lymphocytes. No immature white cells have been found in the circulation.

We can now correlate the general development of the black-eyed whites with the condition of their blood. It seems that the erythrocyte and haemoglobin values shortly before birth are adequate for intra-uterine development. As a consequence, the animals are born with practically normal weights. The oxygen requirements are obviously greater after birth. Hence the reduced haemoglobin, which was just about adequate for the intra-uterine life, is now inadequate to meet the increased requirements, and as a consequence the black-eyed whites fall back during the first few days after birth. Then the general improvement of their erythron enables them to keep pace again with their normal litter mates, and often even to recover the ground lost.

BLACK-EYED WHITES UNDER REDUCED AIR PRESSURE

It was pointed out above that in their general development, the black-eyed whites (W^2) are always more or less subnormal, though sometimes only to a very slight extent. This might be interpreted as indicating that these anemic animals are living precariously on the limit of their adaptability; that they have to use the whole of their adaptive latitude to be just able to develop approximately normally. If this interpretation is correct, one should expect that they would be unable to meet extra demands on their erythron. Under such conditions, there should be a striking difference between the black-eyed whites and their normal litter mates.

An extra strain is imposed upon the erythron at high altitudes. The rarefication of the available oxygen is compensated for by a normal animal by an increase in the oxygen-carrying power of the blood; erythrocytes and haemoglobin become increased. Experiments were therefore performed to investigate whether black-eyed whites would behave differently from normal mice under such conditions.

The mice were kept in desiccators connected to a suction pump. An air leak through a fine capillary provided an air stream running through the apparatus. The pressure was first regulated by varying the suction of the pump, which was fairly constant. Later, a manostat (needle valve mechanism regulated by a spring of variable tension) was introduced as an im-

provement, and this apparatus remained steady within one mm Hg pressure, apart from fluctuations of atmospheric pressure, which were compensated whenever necessary. The animals were fed on their ordinary diet of rat biscuits, wheat and oats, and obtained a liberal allowance of fresh carrots every day as a source of moisture. A big wad of wet cotton wool as an additional water source to moisten the air was later put into the bottoms of the desiccators.

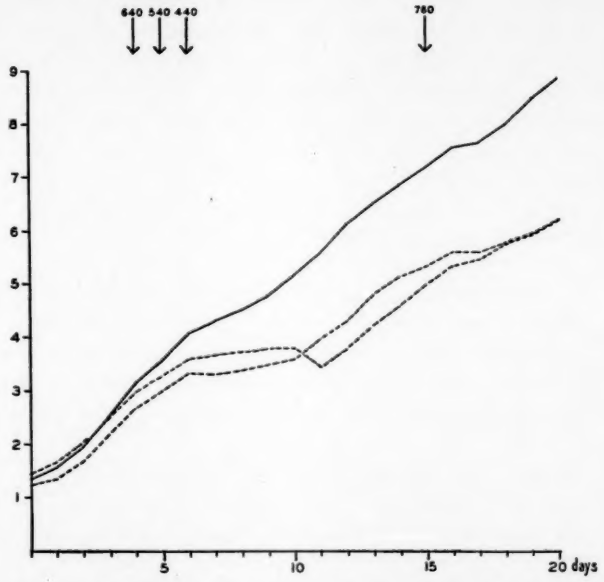

FIGURE 3.—Acclimatization to low atmospheric pressure. Individual growth curves of two W^2W^2 anemics and compound growth curve of eight normal litter mates.

The results obtained varied with the age at which the acclimatization of the litters was started. A typical growth curve (weighings every 24 hours) is given in figure 3. Acclimatization was started at the age of four days. The normals were at first slightly slowed down in their development, but they soon resumed their normal rate of growth. The black-eyed whites were almost completely arrested for several days. Then, about the 10th to 11th day of life, they started growing again and then grew at about the same rate as their normal sibs. On the 15th day of life, the litter was brought back to normal pressure. No further acceleration of growth occurred, showing that complete acclimatization had taken place. Essentially similar results were obtained with two other litters, with which similar experiments were carried out.

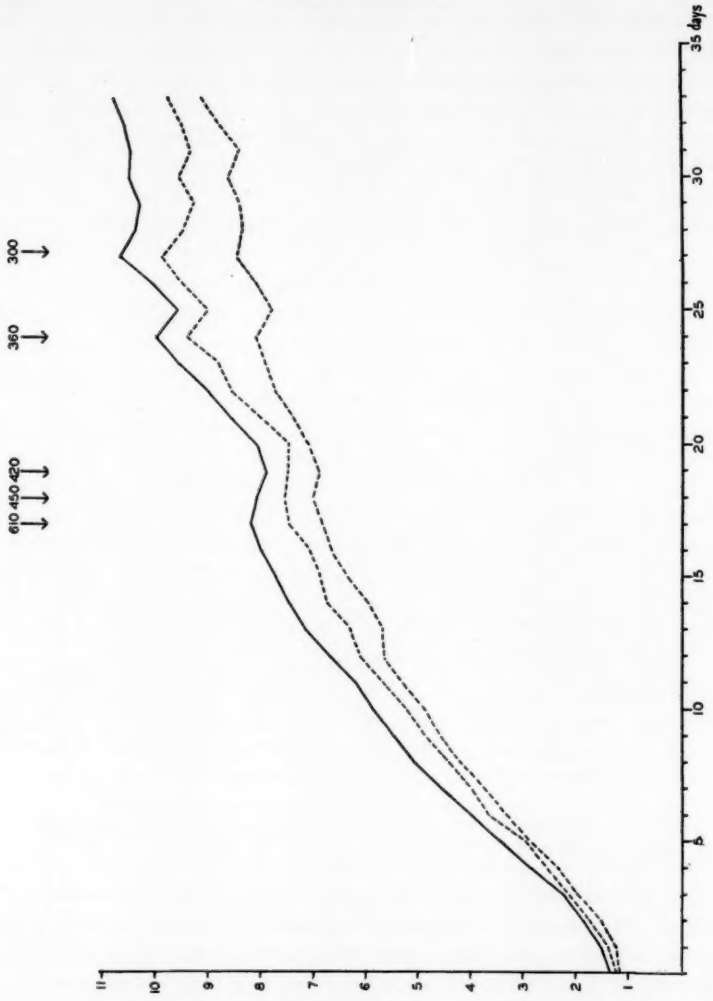

FIGURE 4.—Acclimatization to low atmospheric pressure. Individual growth curves of two W^3W^3 anemics and compound growth curve of eight normal litter mates.

The experiment may be interpreted in two ways. It may be that it always takes about four days before a black-eyed white can start acclimatizing itself to low pressure, irrespective of the time at which the experiment is done, whereas normal animals can do so very much more quickly. Alternatively, it may be that black eyed whites cannot adapt themselves

readily before a critical period which is round about the 10th day of life. It will be remembered that a critical period at about that time was shown to exist by two other independent facts. It was found that the red blood picture of untreated W^2W^2 animals improves noticeably at this time. On the other hand, about one half of the W^1W^2 compounds take a turn for the worse at that age. It therefore seems more likely that black-eyed whites cannot acclimatize themselves before the increased activity of their bone marrow has set in. This is confirmed by the following experiment.

Acclimatization was started in a litter (figure 4) at the age of 17 days. The normals showed a slight decline at the beginning of the experiment and two similar reverses later on, when the pressure was still further reduced. Eventually, at a pressure of only 300 mm of mercury, the limit of adaptability of the normals was practically reached, growth being almost arrested. The two black-eyed whites behaved in exactly the same manner as their normal sibs, and reached the limit of their adaptability at the same pressure. They showed that in no way were they any worse off than the rest.

These experiments show clearly that the black-eyed whites (W^2W^2) are by no means living at or very near the limit of their adaptive latitude. They seem to be able to go to just the same limit of adaptation as their normal litter mates, though this process is delayed if an attempt at acclimatization is made before their period of increased bone marrow activity.

This general conclusion is confirmed by haematological examination of black-eyed whites after adaptation to reduced pressure. Animals from two litters, born on the same day, were acclimatized, starting at the age of 28 days. The pressure was reduced in the following steps: 560, 500, 450, 400 mm Hg (one day for each step); the animals remained at 400 mm Hg for three days, then the pressure was reduced to 350 mm Hg for another six or seven days respectively. Normal and black-eyed white control animals from each litter were kept at atmospheric pressure and examined on the same day as the acclimatized animals. The results are given in table 16.

It is obvious that in both experiments, the acclimatized normals and black-eyed whites have markedly increased the number of erythrocytes per mm^3, the haemoglobin values and the number of reticulocytes per mm^3. Both groups of acclimatized animals exceed the normal range of values quite significantly. But still the black-eyed whites lag behind their normal sibs, doing their acclimatization on a lower level. Averaging the figures for the two pairs of animals and comparing the indices B/N for erythrocyte numbers, haemoglobin, color index and reticulocytes with those of untreated animals of the same age group (taken from tables 5–8), the values of table 17 are obtained.

TABLE 16

Acclimatized (A) and Control (C) animals (N = Normal, B = Black-eyed white).

LITTER	AGE	C OR A	N OR B	Hb %	ERYTHROCYTES PER MM³	COLOR INDEX	RETICULOCYTES PER MM³	RETICULOCYTES %
8/9d	40	C	N	90	9,250,000	.49	304,000	3.29
		C	B	62	5,030,000	.62	289,000	5.75
		A	N	112	13,140,000	.43	940,000	7.15
		A	B	104	8,630,000	.60	924,000	10.71
10/11d	41	C	N	98	10,300,000	.48	472,000	4.58
		C	B	82	7,770,000	.53	542,000	6.98
		A	N	123	13,110,000	.47	1,027,000	7.83
		A	B	97	8,470,000	.57	921,000	10.87

The somewhat surprising result is, that, far from doing worse than their normal litter mates, the black-eyed whites are actually somewhat better off, as compared with their sibs, so far as the haemoglobin and the reticulocytes are concerned, whereas as regards the cell number, they are holding their own. The difference in the color indices is probably without significance. In other words, the difference as to cell numbers has remained the same, although it has been raised to a higher level. A slight relative improvement has taken place under reduced pressure as to the haemoglobin, but the difference is so small that it may be accidental.

It was desired to ascertain whether a black-eyed white, which has increased its cell number and haemoglobin under reduced air pressure, is able to maintain, at least in part, the high level when brought back to atmospheric pressure. One anemic and a normal litter mate (not included in table 16) where therefore kept alive, and erythrocyte counts were made from the tail immediately after the conclusion of the acclimatization experiment, and again two and four days afterwards. Care was taken by means of ligatures to prevent an uncontrolled loss of blood from the cut surfaces. The figures are given in table 18. The experiment shows clearly

TABLE 17

B/N values of acclimatized and untreated animals.

	HAEMOGLOBIN %	ERYTHROCYTES PER MM³	COLOR INDEX	RETICULOCYTES PER MM³	RETICULOCYTES %
Untreated	.79	.66	1.20	.86	1.30
Acclimatized	.86	.65	1.31	.94	1.44

TABLE 18

Erythrocyte counts after the conclusion of acclimatization.

LITTER 8/9d	NORMAL	BLACK-EYED WHITE	B/N
After 0 days	14,110,000	10,410,000	.74
After 2 days	10,575,000	6,615,000	.63
After 4 days	9,940,000	6,325,000	.64

that already after two days, the erythrocyte values have practically reverted to a normal level in both animals. The effort made by the anemic has therefore no more lasting effect than that of the normal animal. No attempts have been made to follow the haemoglobin after the conclusion of acclimatization experiments. It is likely that it behaves in a similar manner.

The experiments described in this section show conclusively that the black-eyed whites (W^2W^2) are by no means living near the limit of their adaptive latitude, but that they can be stimulated successfully to form more cells and more haemoglobin. The differences as compared with their normal sibs remain the same, though on a higher level. When acclimatized animals are brought back to normal pressure, the blood values return to normal figures very quickly, and the anemics are evidently unable to maintain, even partially, the high values which they have reached under strain.

RECOVERY OF W^2 MICE FROM BLOOD LOSSES

It was shown in a preceding section that W^2 anemics spontaneously improve their blood pictures during and after the second week of life. It was further shown that W^2 anemics can be stimulated by reduced air pressure to form more erythrocytes and more haemoglobin than they usually do, so that they actually reach about the same values as a normal mouse under normal pressure. These two facts speak forcibly against an aplastic nature of this anemia. However, it seemed desirable to confirm this conclusion by another independent test. A normal animal which has become anemic due to repeated blood losses is able to re-establish normal blood values fairly rapidly after the blood losses have come to an end. An animal suffering from an aplastic anemia should be unable to do so. An experiment to test the behavior of W^2 anemics after repeated blood losses was therefore undertaken

Two normal and two black-eyed white males of the same litter, 55 days old at the beginning of the experiment, were used. One animal of each kind was bled from the tail on the 1st, 4th, 6th, 8th, 10th, 12th, and 14th day of the experiment; about .25 cc of blood was withdrawn each time, and further uncontrolled blood loss was obviated by means of ligatures.

Examinations of the blood were carried out on the 8th and 14th day of the experiment, by which time a severe secondary anemia had been produced in both animals. The animals were then allowed to recover for 13 days, when a final examination of the blood was made. The two control animals were examined on the 8th, 14th and 27th day. The results are given in table 19. For sake of brevity, this table does not include the color index,

TABLE 19

Recovery from blood losses.

	NORMALS			BLACK-EYED WHITES		
	CONTROL	EXPERIMENTAL	E/C	CONTROL	EXPERIMENTAL	E/C
Erythrocytes per mm³	11,630,000	9,285,000	.80	6,450,000	5,510,000	.85
	11,515,000	7,580,000	.66	7,140,000	4,005,000	.56
	11,300,000	12,330,000	1.09	7,100,000	7,445,000	1.05
Haemoglobin %	105	90	.86	84	67	.80
	100	67	.67	80	50	.63
	101	99	.98	87	86	.99
Volume of packed cells %	51.67	43.33	.84	41.67	35.22	.85
	48.33	37.17	.77	37.83	27.17	.72
	48.67	48.83	1.00	42.17	43.05	1.03
Reticulocytes %	2.53	12.74	5.04	3.29	18.77	5.71
	6.28	22.12	3.52	10.47	18.83	1.80
	3.47	5.48	1.58	5.03	5.75	1.14
Reticulocytes per mm³	294,000	1,183,000	4.02	212,000	1,034,000	4.88
	723,000	1,677,000	2.32	749,000	754,000	1.01
	392,000	676,000	1.40	357,000	428,000	1.20
Leucocytes per mm³	11,133	6,311	.57	5,555	7,800	1.40
	22,600	3,844	.17	12,911	4,378	.34
	13,489	5,089	.38	10,178	9,289	.91

mean erythrocyte volume and mean corpuscular haemoglobin concentration per unit volume of cells, which can be calculated readily from the data presented. The three values given for each item represent the state of affairs on the 8th, 14th, and 27th day of the experiment respectively. Strictly speaking, the controls are valid only for the 8th day, because afterwards, they had lost blood on two occasions. It seems, however, that this has not altered their blood pictures to any appreciable extent, except that the increase in reticulocytes is probably due to these blood losses.

Considering first the blood counts, haemoglobin values and the volume of packed cells percent, it is evident from the ratio of experimental to control values (E/C) that a mild anemia had been produced after one

week; after a fortnight, the induced anemia had become very severe, and somewhat more pronounced in the black-eyed white than in the normal animal. After a period of another 13 days, however, both the normal and the black-eyed white have completely recovered; as to erythrocyte numbers, both animals seem to have actually overshot the mark, though only to a slight extent. The animals subjected to blood losses both show a very marked reticulocyte response, relatively as well as absolutely. Normal values had not yet been reached again at the conclusion of the experiment.

The experiment shows clearly that the black-eyed white, like a normal mouse, can recuperate completely from an anemia due to blood losses. Its blood-forming organs are able to react to this stimulus. The anemia cannot be aplastic.

LIVER TREATMENT OF W^2 ANEMICS

In man, most macrocytic anemias, notably pernicious anemia, react favorably to liver treatment. It was therefore of interest to ascertain whether the macrocytic anemia of W^2 mice could be influenced in a similar way. Two litters, 30 and 54 days old at the beginning of the experiment were treated. One normal and one black-eyed white of each litter were injected subcutaneously with 0.5 cc of "Liver Extract B.D.H. (British Drug Houses Ltd.) for intramuscular injection" every other day for a period of 22 days. One normal and one black-eyed white of each litter served as controls and were injected with 0.5 cc of 0.93 percent NaCl solution. Erythrocyte and reticulocyte counts were made from tail blood every other day during the first fortnight, and again on the 18th day. In view of the negative evidence as presented below, these data are not given here. On the 22nd day of the experiment, the animals were sacrificed and complete blood pictures were obtained. The result is given in table 20; the upper half of this table refers to the younger, the lower half to the older litter. Comparison of the data shows that no noticeable effect on any constituent of the blood has been produced by the liver injections. It is a curious fact that the leucocyte counts in all four pairs of animals are higher in the anemics than in their normal sibs. It cannot be decided from these data whether this is due to the injections as such, irrespective of the substance injected, or to the repeated bleeding, or to a mere coincidence.

A similar experiment was performed with "Campolon," a liver extract for intramuscular injection produced and kindly put at my disposal by the I. G. Farbenindustrie, Leverkusen, Germany. A litter containing W^2 mice was used which was 43 days old at the beginning of the experiment. The treatment lasted for 16 days, during which time two black-eyed whites and one normal animal received five subcutaneous injections of .5 cc of

Campolon each, while two other black-eyed whites and one normal served as uninjected controls Unlike the "Liver Extract B.D.H.," Campolon was tolerated by the mice with difficulty; following the injections, the animals were obviously very uncomfortable for several hours. The animals were examined once only at the conclusion of the experiment. The result was entirely negative and will not be given here in detail; no

TABLE 20

Injection of Liver Extract B.D.H.—Close of experiment.

	CONTROL			EXPERIMENTAL		
	N	B	B/N	N	B	B/N
Erythrocytes	11,780,000	5,625,000	.48	10,575,000	5,575,000	.53
Haemoglobin %	100	70	.70	90	65	.72
Cell Volume %	46.67	37.17	.80	46.67	33.47	.72
Color Index	.42	.62	1.48	.43	.58	1.35
Cell Volume μ^3	39.62	66.07	1.67	44.13	60.03	1.36
Haemoglobin Concentration %	29.57	25.99	.88	26.61	26.80	1.01
Reticulocytes %	3.47	6.54	1.88	6.28	8.00	1.27
Reticulocytes per mm³	409,000	368,000	.90	664,000	446,000	.67
Leucocytes	1,918	2,422	1.26	3,867	5,089	1.32
Erythrocytes	10,940,000	6,395,000	.58	12,160,000	6,460,000	.53
Haemoglobin %	100	80	.80	99	84	.85
Cell Volume %	48.33	38.00	.79	46.67	39.67	.85
Color Index	.46	.63	1.37	.41	.65	1.59
Cell Volume μ^3	44.18	59.42	1.34	38.38	61.43	1.60
Haemoglobin Concentration %	28.55	29.32	1.03	29.27	29.22	1.00
Reticulocytes %	5.03	7.32	1.46	4.21	7.58	1.80
Reticulocytes per mm³	550,000	468,000	.85	512,000	490,000	.96
Leucocytes	2,133	5,867	2.75	3,262	4,200	1.29

effect was noticeable on either haemoglobin, red cell count, volume of packed cells per unit volume of blood, or the color index, the mean erythrocyte volume and the mean corpuscular haemoglobin concentration derived from these figures. The reticulocytes, both as a percentage and as absolute figures, tended to be lower in the injected than in the control animals, but this difference is of doubtful significance; similarly, the leucocyte counts were somewhat lower in injected animals than in their controls.

Neither "Liver Extract B.D.H." nor "Campolon" is a highly purified substance. They were selected for this very reason, as in highly purified substances, there is always the risk that a potentially potent constituent

of raw liver may have been eliminated in the process of purification. The completely negative results obtained with both substances cannot be due to insufficient dosage or time of treatment. The conclusion is therefore that W^2 anemics (and presumably W^1 anemics and compounds as well) do not react to the Pernicious Anemia Principle contained in liver.

THE HAEMATOLOGY OF W^1W^2 COMPOUNDS

W^1W^2 compounds were obtained by crossing commercial W^1wss (black-eyed white) females to W^2wSS males. As expected, they were recognizably anemic at birth and later became black-eyed whites. The data on these compounds are not very extensive, but taken in conjunction with the

TABLE 21

W^1W^2 stock. Haemoglobin values, erythrocyte counts, color indices and reticulocyte counts.

LITTER	AGE IN DAYS	NORMALS					BLACK-EYED WHITES				
		Hb %	ERYTHROCYTES PER MM³	COLOR INDEX	RETICULOCYTES PER MM³	%	Hb %	ERYTHROCYTES PER MM³	COLOR INDEX	RETICULOCYTES PER MM³	%
44a	0	101	6,135,000	.82	3,902,000	63.60	50	1,645,000	1.52	1,344,000	81.68
44a	0	88	4,650,000	.95	2,976,000	64.00	54	1,920,000	1.41	1,405,000	73.20
41a	1	69	4,435,000	.78	1,394,000	31.44	34	980,000	1.73	542,000	55.28
41a	1	62	2,750,000	1.13	1,023,000	37.20	31	1,005,000	1.54	604,000	60.06
41a	3	55	2,415.000	1.14	650,000	26.90	26	825.000	1.58	178,000	21.60
41a	3	56	2,835,000	.99	907,000	32.00	30	1,240,000	1.21	285,000	22.98
13c	4	72	6,370,000	.57	2,001,000	31.41	26	1,275,000	1.02	396,000	31.08
13c	4	72	5,950,000	.61	1,480.000	24.87	23	1,235,000	.93	267,000	21.63
16c	6	57	3,940,000	.72	1,490,000	37.81	31	1,765,000	.88	680.000	38.54
16c	6	75	6,065,000	.62	1,742,000	28.72	31	1,575,000	.98	699,000	44.38
16c	8	73	5,785,000	.63	1,602,000	27.69	30	1,635,000	.92	693,000	42.40
16c	8	65	4,610,000	.71	1,573,000	34.12	30	1,415,000	1.06	548.000	38.73
12b	10	70	6,350,000	.55	1,528,000	24.07	45	3,215.000	.70	954,000	29.68
12b	10	67	6,500,000	.52	1,430,000	22.00	32	1,770,000	.90	598,000	33.77
13d	16	68	6,330.000	.54	539,000	8.51	40	2,875,000	.85	425,000	14.77
12a	18	66	5,250,000	.63	1,252,000	23.84	46	3,610,000	.64	1,022,000	28.32
12a	19	62	5,380,000	.58	878.000	16.32	17	1,190,000	.72		
16a	21	60	5,640,000	.53	627,000	11.11	42	3,170,000	.67	495,000	15.61
16a	22	63	6,390,000	.49	1,208,000	18.90	42	3.240,000	.65	627,000	19.35
16a	25	81	7,750,000	.52	1,870,000	24.13	50	3.680.000	.68	854,000	23.20

material on the W^2W^2 anemics, the interpretation is fairly clear. The individual figures are given in table 21, the various averages together with their B/N values are found in table 22.

It will be noticed that the general trends in the development of the W^1W^2 compounds correspond very well to those established for the W^2W^2 anemics. The anemia is hyperchromic. There is an improvement both in erythrocyte number and in haemoglobin percentage after the tenth day of life, and the reticulocytes, both expressed as a percentage of all red cells and in absolute figures, behave very similarly. There is therefore a good qualitative agreement between the two types. The differences are found by quantitative comparison. The B/N values for erythrocytes and haemoglobin are markedly lower in W^1W^2 than in W^2W^2 anemics. For all animals

up to the age of 10 days, the B/N values for erythrocytes are .31 and .50 respectively, and for haemoglobin the values are .48 and .65 respectively. The color indices tend to be higher in W^1W^2 than in W^2W^2. It appears that, unlike the W^2W^2 anemics, the color index (B/N) goes down with age. To what extent this trend is significant is difficult to decide. The author is inclined to regard the color index in the first age group as unduly high; if so, the gradient would be less steep than it appears from the raw figures.

TABLE 22

Averages and B/N values for W^1W^2 anemics and normal sibs.

AGE IN DAYS	N OR B	HAEMOGLOBIN %	ERYTHROCYTES PER MM³	COLOR INDEX	RETICULOCYTES PER MM³	RETICULOCYTES %
0– 1	N	80.0	4,492,500	.89	2,323,806	51.74
	B	42.3	1,387,500	1.52	973,606	70.17
	B/N	.53	.31	1.71	.42	1.36
3– 4	N	63.8	4,392,500	.73	1,259,354	28.67
	B	26.3	1,143,750	1.15	281,638	24.62
	B/N	.41	.26	1.58	.22	.86
6–10	N	67.8	5,541,667	.61	1,568,454	28.30
	B	33.2	1,895,833	.88	695,605	36.69
	B/N	.49	.34	1.43	.42	1.30
16–25	N	66.7	6,123,333	.54	1,062,115	17.35
	B	41.0	2,927,500	.70	684,505	20.65
	B/N	.61	.48	1.30	.65	1.19

The color index in the last age group is presumably low as the result of selection. Only a minority of W^1W^2 anemics reach this age group, and it may be assumed that those animals will mainly survive in which the pathological process—whose expression the color index is—is least pronounced.

Comparison between tables 4 and 21 shows another difference. W^2W^2 anemics behave very uniformly, apart from the changes connected with age. In table 21, there occur striking differences between litter mates of the same age. In the 10 days old anemics, the cell counts differed very much (3,215,000 and 1,770,000), and there was a correspondingly great difference in weight (5.53 and 4.31 grams respectively). It appears virtually certain that the stronger specimen with the high cell count belonged to that group of anemics which is relatively long-lived, whereas

the other individual would presumably have died shortly. Similarly, there is a great difference in the counts between the litter mates of 18 and 19 days (3,610,000 and 1,190,000 cells) and a striking difference in the haemoglobin values (46 and 17). Again, the animal with the better blood values was vigorous, while the other specimen was extremely miserable and had developed diarrhoea. The averages for the W^1W^2 compounds are therefore to be taken with greater caution than those of the more homogeneous W^2W^2 groups.

It seems that under certain circumstances, which are but rarely realized, an occasional W^1W^2 individual can improve its blood picture such as

TABLE 23

Blood pictures of a long-lived W^1W^2 and a normal sib.

	69 DAYS OLD			100 DAYS OLD		
	N	B	B/N	N	B	B/N
Erythrocytes	10,400,000	6,815,000	.66	11,255,000	6,440,000	.57
Haemoglobin %	104	86	.83	108	84	.78
Cell Volume %	52.50	46.00	.88	57.00	38.33	.67
Color Index	.50	.63	1.26	.48	.65	1.35
Cell Volume μ^3	50.48	67.50	1.34	50.64	59.52	1.18
Haemoglobin Concentration %	27.34	25.80	.94	26.15	30.24	1.16
Reticulocytes %	1.67	1.96	1.17	1.96	4.03	2.06
Reticulocytes per mm³	174,000	134,000	.68	221,000	260,000	1.18
Leucocytes	6,864	4,778	.70	6,489	9,356	1.44

to practically equal the condition found in adult W^2W^2 mice. It was mentioned above that one out of eleven compounds whose life history was followed completely, proved very long-lived and vigorous. Blood pictures of this female and a normal sister were obtained at the ages of 69 and 100 days. They are given in table 23. It is obvious that in every respect, this animal was equal to a W^2W^2 mouse of the same age.

It appears that the compounds have a very much worse start in life. The majority do not survive and fall victims to inter-current diseases. Sometimes an animal will survive the critical period and may then become practically as good as a W^2W^2 mouse Whether a secondary deterioration of the blood picture may occur in W^1W^2 cannot be decided from the material available.

It remains to add a few data about blood smears in the compounds. The smears are on the whole very similar to those found in W^2W^2; there are no normoblasts or megaloblasts, and immature white cells have not been observed. The anisocytosis is more pronounced in animals with low

blood counts than in those with higher counts. A few distributions of red cell diameters have been included in table 9. The mean cell diameters of 250 cells at 10 days of age and their coefficients of variation are:

NORMAL		BLACK-EYED WHITES		B/N
M.C.D.	v	M.C.D.	v	
6.908±.052	11.90%	7.862±.059	11.86%	1.138
		8.090±.068	13.36%	1.171

The B/N values are somewhat higher than in W^2W^2 animals; this was to be expected as the color indices are also higher. The black-eyed white with the bigger cells had a higher color index than that with the smaller cells.

DISCUSSION

We can now compare the three related anemias W^1W^1, W^1W^2 and W^2W^2. For W^1W^1, DE ABERLE'S (1927) detailed account is available. Only new-born animals can be compared, as the untreated W^1W^1 anemic survives only for a few days. The B/N values for erythrocytes, haemoglobin and color indices are compared in table 24.

TABLE 24

B/N values for new-born W^1W^1, W^1W^2 and W^2W^2 anemics.

	ERYTHROCYTES PER MM³	HAEMOGLOBIN %	COLOR INDEX
W^1W^1	.14	.25	1.79
W^1W^2	.31	.53	1.71
W^2W^2	.51	.66	1.29

As regards the erythrocytes, the compound is very nearly intermediate between the two pure types. As to haemoglobin, the compound is nearer W^2, and as a consequence the color index is nearer W^1. As pointed out above, it may be that the haemoglobin values of the compounds, based on only four pairs of individuals of that age, were accidentally too high, and that therefore all the three values would in reality be approximately intermediate. In the behavior of the reticulocytes, all the three types behave alike. It was found by DE ABERLE that the W^1W^1 anemics are late in replacing reticulocytes by fully mature erythrocytes. The same occurs in W^1W^2 and in W^2W^2 anemics. The size of the W^1W^1 erythrocytes was not investigated in detail by DE ABERLE. A few haematocrit values obtained by the author leave no doubt that that anemia is also of a macrocytic character. W^1W^1 anemics have a reduction of leucocyte numbers, but this is less pronounced than the reduction of red cells. The same situation is found in W^2W^2 anemics. The three anemias therefore agree in all essential points and differ only by degree.

Aetiologically, it is clear at once that the *W* anemias are not due to loss of blood from the circulation. If haemorrhages occurred, their site should be found in post-mortem examinations; no sign of haemorrhage has been discovered in any of the numerous post-mortems that have been carried out. Besides, a secondary anemia due to bleeding would be microcytic with a lowered color index.

It is less easy to decide whether the anemia of the *W* mice is due to increased destruction of erythrocytes in the circulation, or whether it is due to faulty production of blood cells. In this connection a detailed study of the inner organs and of the bone marrow would be helpful. The color of the serum of W^2 mice (50 and 145 days old) did not differ from that of their normal sibs. Fouchet's test for pathologically increased bilirubin in the serum was negative in all four animals. However, Fouchet's test is not very sensitive, and if the mouse is able to rid itself easily of surplus bilirubin, even a completely normal bilirubin level in the serum would not necessarily exclude haemolysis as a contributing factor to the anemia of *W* mice.

However, it seems more likely that the main, and possibly the whole reason for the anemia is a fault in blood production. In typical haemolytic anemias, there usually is an increase, both relatively and absolutely, of the reticulocytes. In the *W* anemics, there may be a relative increase, but the total number is always reduced. This points clearly in the direction of faulty production.

In a recent paper, BRUNER, VAN DE ERVE and CARLSON (1938) have described the normal blood pictures of young rats from birth to 24 days of age. They point out that this period in the rat is the transition period from the megaloblastic erythropoiesis of the fetus to the normoblastic erythropoiesis of the adult rat. A new-born rat—and the same is true for a new-born mouse—when compared with an adult individual, shows every sign of a macrocytic anemia: it has less haemoglobin and a smaller number of erythrocytes per unit volume of blood, but the size of the cells is considerably greater; the color index is higher, but the haemoglobin concentration per unit volume of cells is substantially the same. In the general trends, though of course not quantitatively, our data on the normal mouse are in excellent agreement with the observations of these authors; mouse and rat behave in almost the same way with very little difference.

It will be remembered that this is precisely the situation as we have described it for the *W* anemics in relation to their sibs in this paper. The conclusion to be drawn is obvious: ***W anemics differ from normal mice by the fact that the transition from the megaloblastic to normoblastic erythropoiesis is delayed and remains incomplete.*** This conclusion is fully compatible with the results of the low pressure experiments. Both kinds of animals react

to the unspecific stimulation, the normals normoblastically and the anemics megaloblastically; the difference persists, but on a higher level. If this interpretation is correct, the *W* anemia is comparable to pernicious anemia in man: in the *W* anemic, the organism never advances completely to normoblastic erythropoiesis; in pernicious anemia, it reverts from normoblastic to megaloblastic erythropoiesis. The negative results of liver treatment are compatible with this interpretation; the *W* anemics may be unable to utilize the P.A. principle contained in liver. It would be interesting to ascertain whether the livers of *W* mice contain that principle. The technical difficulties in preparing such extracts and testing them on pernicious anemia patients would be considerable.

In her analysis of the W^1 anemia, DE ABERLE came to the conclusion that that anemia was of an aplastic nature. In the light of the facts presented in this paper, I cannot agree to this conclusion. In an aplastic anemia, the regenerative powers of the erythron are impaired; there always is a reduction of reticulocytes both absolutely and relatively. In W^1W^2 and W^2W^2 mice, the relative number of reticulocytes is at times strikingly increased. A true aplastic anemia should always be progressive, as the erythron is unable to replace the normal wastage of cells. The W^2W^2 anemics are not only able to maintain the *status quo*, but they manage to improve their condition considerably; they are able to react to unspecific stimulation by producing more cells. All that would be impossible in case of an aplastic anemia. As the W^2 anemia differs from the W^1 anemia only by degree, not fundamentally, this reasoning applies to W^1 anemics also.

It remains to compare the *W* anemia with the other inherited anemias described in rodents. The transitory anemia associated with flexed-tail in the mouse (MIXTER and HUNT 1933; KAMENOFF 1935) is hypochromic and of unknown aetiology; a more detailed investigation would be of considerable interest. The inherited acholuric jaundice in rats described by GUNN (1938) is haemolytic in nature. The progressive lethal anemia in the rat recently described by SMITH and BOGART (1939) is microcytic and differs in many ways from the *W* anemias.

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The author is greatly indebted to DR. C. C. LITTLE and DR. A. M. CLOUDMAN for their generosity in supplying him with W^2 mice. He has to thank PROF. J. B. S. HALDANE for the interest he took in the work throughout, and DR. M. MAIZELS (University College Hospital, London) for repeated help and advice. Most of the blood counts were carried out by DR. DORA BAK, to whom I am greatly obliged.

SUMMARY

1. Mice homozygous for W^1 are born with considerably reduced weights; mice homozygous for W^2 have practically normal birth weights, and W^1W^2 young are intermediate between the two pure types.

2. W^1 homozygotes are unable to put on weight. W^2 homozygotes grow up almost, but not quite, normally. W^1W^2 mice grow at a reduced rate for about nine days; most of them die within the first six weeks; occasionally a compound survives to adult age.

3. Haematologically, young W^2 anemics are characterized by a reduction of erythrocytes to about one half and of the haemoglobin to about two thirds of the normal values. The color index is raised, but the mean haemoglobin concentration per unit volume of cells is normal. The mean erythrocyte diameter and volume is increased. The resistance of the red cells to hypotonic salt solutions is normal. There are neither nucleated red nor immature white cells in the circulation. The reticulocytes are, at most age groups, relatively increased, but always absolutely reduced in numbers. The white cells are reduced to a lesser extent than the red cells. During the second week, the W^2 anemics improve their condition as compared with the normals to some extent, but never reach normal values.

4. Under reduced air pressure, both normals and W^2 mice are able to increase erythrocyte numbers and haemoglobin values. The difference between normals and anemics persists, however, but on a higher level. On return to normal pressure, the counts return to normal values equally quickly in normal and anemic animals. If an attempt at acclimatization is made with very young anemics, the acclimatization is delayed.

5. W^2 anemics are able to recover from repeated blood losses like normal mice.

6. Treatment of W^2 anemics with "Liver Extract B.D.H. for intramuscular injuction" proved ineffective.

7. The haematology of W^1W^2 compounds is qualitatively very similar to that of W^2W^2 mice, but the anemia in all its aspects is more severe. It is very nearly intermediate between that of W^2W^2 and W^1W^1 as described by DE ABERLE. A very vigorous and long-lived individual had a blood picture practically indistinguishable from that of W^2W^2 mice of the same age.

8. W mice differ from normal mice by the fact that the transition from the megaloblastic erythropoiesis of the fetus to the normoblastic erythropoiesis of the adult is delayed and remains incomplete.

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AN ANALYSIS OF THE CHROMOSOMES OF THE TWO SUB-SPECIES DROSOPHILA VIRILIS VIRILIS AND DROSOPHILA VIRILIS AMERICANA*

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INTRODUCTION

INCREASING attention is being focused on the use of Drosophila as a convenient organism for investigating such fundamental problems as the formation of new species and interspecific sterility. Studies of the hybrid from the cross *D. melanogaster*×*D. simulans* by PÄTAU (1935), and by HORTON (1939), and *D. pseudoobscura*×*D. miranda* by DOBZHANSKY and TAN (1936), as well as studies of the hybrids from the interracial crosses of *D. pseudoobscura* by DOBZHANSKY and STURTEVANT (1938), have amply demonstrated the advantages of the salivary gland technique in affording a new approach to such old problems, and making possible a more critical analysis of the differences in gene alignment.

The present paper is a study of the differences in gene alignment of the two sub-species, *D. virilis virilis* and *D. virilis americana* as determined by the salivary gland chromosome analysis of the hybrid, and the differences in the chromosome configurations in the larval ganglion cells. Such a study has made necessary a revision of my preliminary map (HUGHES 1936) of the salivary gland chromosomes of *D. virilis*.

These two sub-species are of special interest because, among other reasons, they can be crossed easily and yield hybrids which are partially fertile (SPENCER 1938). Both male and female hybrids are partially fertile when crossed *inter se*, or backcrossed to either parent sub-species. That two sub-species differing so widely in gene alignment, in the chromosome configuration of the ganglion cells, in phenotypic appearance, and physiological characteristics can be crossed is unusual for the genus Drosophila, and it is remarkable indeed that the hybrid is partially fertile. (Recently it has been reported that the female hybrid from the cross *D. miranda*×*D. pseudoobscura* is slightly fertile under certain conditions) (MACKNIGHT 1939). The fortunate occurrence of partial fertility in the hybrids obviously will make it possible to follow the behavior of the genetic complexes of the two sub-species in subsequent generations.

I am deeply indebted to PROFESSOR WARREN P. SPENCER of the College of Wooster for calling my attention to *D. virilis americana*, as well as for sending me stocks. To PROFESSOR FRANZ SCHRADER of Columbia Univer-

* The cost of the accompanying plate has been contributed by the Galton and Mendel Memorial Fund.

sity, under whose guidance this work was done, I wish to express my thanks for his unfailing interest and many helpful suggestions.

MATERIALS AND METHODS

Source of Stocks. The wild type strain of *Drosophila virilis virilis* used in this study as the standard is a Chinese strain (China-a) obtained through the Carnegie Institution of Washington, Cold Spring Harbor, New York. Preliminary observations of the salivary gland chromosomes of F_1 larvae obtained when the Chinese strain is crossed with American (New Orleans), or Japanese strains obtained from the same source, failed to reveal any obvious differences among the chromosomes of the three strains.

The strain of *D. virilis americana* used was started from a single impregnated female collected by W. P. SPENCER in Wayne County, Ohio. Three other *americana* females have since been collected by W. P. SPENCER and H. D. STALKER in Gatlinburg, Tennessee (written communication).

Description of Phenotypes. D. virilis americana differs from *D. virilis virilis* in a number of distinctive characteristics. It has a much larger eye, with a finer eye pile; broader carina; darker body color; heavier cloud on posterior cross-vein; and a more fusiform body. *Americana* tends to pupate in the culture medium rather than on paper or on the side of the culture bottle, and the pupa cases are reddish rather than gray. The time required for anesthetization is less for *americana* (SPENCER 1938). In addition, the larvae are generally smaller in *americana*, and the salivary gland preparations are generally inferior in staining quality. There is some evidence that the cells (for example, the ganglion cells) of the larvae of *americana* are generally smaller, although careful measurements have not been made to determine this point accurately.

Larvae. Hybrid larvae from the cross *virilis* ♂×*americana* ♀, and the reciprocal cross, are easily obtainable. SPENCER reports that approximately 2 percent of the eggs develop into larvae when *virilis* ♂'s are crossed with *americana* ♀'s. I have made no attempt to determine the exact percentage of eggs which develop when various crosses are made, but certain facts concerning the extent of fertility have been observed. The above crosses may be made with wild type individuals of both sub-species, but strikingly enough it appears that when certain strains of *virilis* are used, the ease with which larvae can be obtained is much greater. My observations on this point are of a preliminary nature only, but when, for example, *virilis* ♀'s with the mutations scute vermilion apricot are crossed to wild type *americana* ♂'s, the percentage of larvae obtained is distinctly greater than from the cross wild type *virilis* ♀'s×wild type *americana* ♂'s. It is not known whether the differences may be accounted for by genetic, or other factors.

Well-fed hybrid larvae are as large and sometimes larger than pure *virilis* larvae. As a rule, *virilis* larvae are approximately a third larger than *americana*. The larval gonads by which the sexes of the larvae are easily distinguished, are correspondingly larger in *virilis*. In the hybrid, the size of the larval gonads approximates that of *virilis*, that is, the testis is a rather large elliptical mass lying dorsally in the posterior third of the body embedded in a fat body, whereas the ovary is a small round body about a sixth the size of the testis, and lying in the same relative position.

The most satisfactory method for obtaining large numbers of hybrid larvae for study was to make a mass culture in half-pint milk bottles, using about a dozen males and a dozen females. The culture medium was banana-agar spread on caps and heavily yeasted. The caps were changed daily and the old food with the eggs was transferred to half-pint milk bottles containing the standard cornmeal-molasses-agar medium to which had been added a generous amount of brewer's yeast. The larvae remained in this medium until ready for use.

Salivary Gland Chromosomes. Various techniques were used in preparing the salivary gland chromosomes for study. The best preparations were obtained by using the following modified aceto-carmine method: The salivary glands from well-fed, fully mature larvae were dissected out in 45 percent acetic acid and allowed to remain in this fixative from 10–15 minutes. After fixation the glands were transferred to clean slides where they were spread between the slide and a cover slip, using a needle to exert a gentle pressure on the cover slip. To facilitate the later removal of the cover slips, they were left with one edge slightly overhanging the slides when the glands were smeared out. The slides, with cover slips in place, were then transferred to a 95 percent alcohol vapor bath for 12–24 hours. After the vapor bath, slides with cover slips intact were transferred to a dish containing 95 percent alcohol, and permitted to remain there for 6–24 hours. The cover slips were then removed by floating off in 95 percent alcohol, or, if necessary, by inverting the slides and exerting a gentle pressure on the overhanging edge of the cover slips. Both slides and cover slips were separately given the following treatment: Dipped several times in 45 percent acetic acid, stained in 45 percent acetic acid supersaturated with carmine for 5–15 minutes, and dipped in two changes of 95 percent alcohol. The cover slip which was removed was then returned to the same slide, and the two were mounted together using euparal as the mounting medium. The chief advantage of this method over the usual aceto-carmine smear method is that the first period of fixation and the staining time can be varied independently of one another. This is important because the conditions one usually strives for are intense contrast of bands and achromatic material on the one hand, and well stretched chromosomes on the other. These conditions are not usually compatible, since the first condi-

tion requires a long period of staining (therefore fixation), and the latter requires a short period of fixation (before the chromosomes are too brittle to stretch out). Moreover, the chromosomes take the stain much more rapidly when exposed directly, with the usual result that the chromosomes are intensely colored while the cytoplasm is barely tinged. Thus chromosomes do not have to lie free from the cytoplasm to be seen clearly.

Ganglion Chromosomes. Larval or pupal ganglia were dissected out in Ringer's solution, fixed in strong Flemming's solution, and stained with iron haematoxylin. All metaphase configurations were drawn from tissues prepared in the above manner. In some instances the ganglia were smeared and stained with aceto-carmine as a control.

Drawings. All drawings were made with the aid of a camera lucida. In the drawings of the ganglion chromosome configurations 25× oculars and 90× objectives were used, and for the salivary gland chromosomes 20× oculars, and either a 90× objective or 110× objective.

Drawings of the normal chromosomes of *virilis* were made from selected sections of various preparations. The drawings are, therefore, composites and represent average, well stretched chromosomes. Particular attention was paid to thickness of the bands, the intensity with which they took the stain, and to the more constant constricted and inflated regions. No attempt was made to represent the coiling of the homologues about one another.

The drawings of the hybrid are not composite, but represent a single pair of chromosomes, each figure from a single nucleus. The figures selected for drawing are perhaps more closely paired than is typical.

SALIVARY GLAND CHROMOSOMES OF THE NORMAL *D. virilis virilis*

As pointed out by HEITZ (1934), FUJII (1936), and HUGHES (1936), the number of elements in the salivary gland nuclei of *D. virilis virilis* is six, the longer elements corresponding to five pairs of rod-like chromosomes of gonial metaphase, and the very short element corresponding to the microchromosomes. All six chromosomes show complete pairing with their homologues in the salivary gland nucleus, except, of course, the X chromosome of the male. Although the present study is based almost entirely on chromosomes from female larvae, many opportunities have arisen in which chromosomes from the male have been observed. In no instance has a Y chromosome been detected in the salivary gland nucleus. Probably the Y is entirely heterochromatic and is thus obscured by the chromocenter region. Further study, however, will have to be made before this point is definitely decided.

In crushing out glands, chromosomes are often broken if the pressure on the cover slip is too great, or if the chromosomes are brittle. These

breaks apparently may occur at any point in a chromosome. Even in comparatively unstretched chromosomes, however, breaks, or greatly attenuated regions, are frequently encountered. This latter type of break does not occur at random throughout a chromosome, but at very definite regions. These regions, which I shall term "weak points" appear to be definitely weaker than other parts of the same chromosome. Almost invariably, if the chromosome is broken, or attenuated, these weak regions will be involved. The frequency with which these weak points are prone to break varies with the region, the range being from a frequency that might be expected by chance to as high as 50 percent or more. These weak points are usually located at constricted regions of the salivary gland chromosomes. It is not known, however, whether or not these weak points correspond to similarly constricted regions in the gonial chromosomes reported in other species, since such regions for the latter chromosomes have not been described for *virilis*. If these weak points have any genetic significance, as for example, the more frequent occurrence of inversion breaks at these points, there is no supporting evidence for this in the study of the hybrid.

Occasionally chromosomes clearly exhibit a 4-partite structure, especially where the homologues happen to be unpaired. No chromosome has yet been observed, however, which was divisible into a greater number of separate parts, as has been reported for some species (FROLOVA 1937), unless we accept the view that each granule or vesicle, many of which constitute a band, represents a chromomere.

The so-called β-heterochromatin (HEITZ), an intensely staining, usually vacuolated structure, is a very conspicuous element of the chromocenter region of *virilis*.

With the aid of chromosomal aberrations, FUJII and HUGHES independently correlated the salivary gland chromosomes with their respective linkage groups. That these interpretations were correct has been further substantiated by additional observations, using new or different aberrations for study.

In the new map of *virilis* the total length of the chromosomal elements has been assigned 100 arbitrary units instead of 101. The new average lengths of the chromosomes in micra are as follows: X (1)—203; B (2)—245; C (3)—190; D (4)—212; E (5)—199; M (6)—11; total 1060 micra. On the basis of these measurements, each of the chromosomes was assigned a definite number of arbitrary units approximately in proportion to their individual lengths as follows: X (1)—1–19; B (2)—20–42; C (3)—43–60; D (4)—61–80; E (5)—81–99; M (6)—100. Each of these major divisions are further subdivided into six smaller units, denoted by capital letters after the scheme of BRIDGES (1935). The scale numbers and letters

run in an ascending series from left to right, that is, from the distal, or free ends of the chromosomes to the proximal, or chromocenter ends. As in the old map, the division lines are placed just to the left of the more conspicuous bands. There are usually several bands in each subdivision. Individual bands may be designated by determining their order in the subdivision. Thus, the second band from the left in the third major division of chromosome 3 is 45A2.

The map of the normal salivary gland chromosomes is shown in Plate I, and a photomicrograph in figure 1(a).

SOME DISTINCTIVE CHARACTERISTICS OF THE INDIVIDUAL CHROMOSOMES

X (1).—The distal end of this unit, from 1A to 1C, has a characteristic frayed-out appearance. In this region the chromomeres tend to be scattered, instead of being organized into sharp bands. In this respect it resembles the heterochromatic region of the chromocenter end, except that the chromatin is less densely aggregated and stains less intensely. Another conspicuous characteristic of the distal end, in region 1E, is what sometimes appears as a very broad, deeply stained band, but in reality is made up of 4–5 sharp bands closely apposed. Region 6, and the vicinity, is interpreted correctly with difficulty. Here the chromomeres are irregularly organized and usually heavily stained. There appears to be a greater tendency for the chromosome to be twisted and distorted in this region, and frequently the general matrix of the chromosome, as well as the chromomeres, takes the stain, thus obscuring the lighter bands. In the region from about 15E to 16B is one of the most distinctive features of the first chromosome. It is typically an enlarged area with several irregular, broken bands, most of which stain very lightly. These characteristics make it difficult to find a constant pattern for this region. The heterochromatin of the chromocenter ends of all the chromosomes, if present, is particularly difficult to analyze. The heterochromatin in the X is no exception to this rule. In favorable preparations this region shows approximately ten heavily stained, thick bands. The chromosomes in this region are typically not organized into distinct bands, and frequently it is with difficulty that they can be identified at all. Under favorable conditions, however, several bands are clearly present. This heterochromatic region extends from 19E1 through 19F.

B (2).—Chromosome 2 is clearly the longest of the salivary gland chromosomes. It is at least 22 times the length of the minute M (6) chromosome. The distal end is readily identified by its dumbbell shape. In the middle of the dumbbell are three dark, double bands, beginning at 20C1. One of the more conspicuous weak points, referred to above, is located in this chromosome at 21C. Region 28A–D is typically inflated, with diffuse

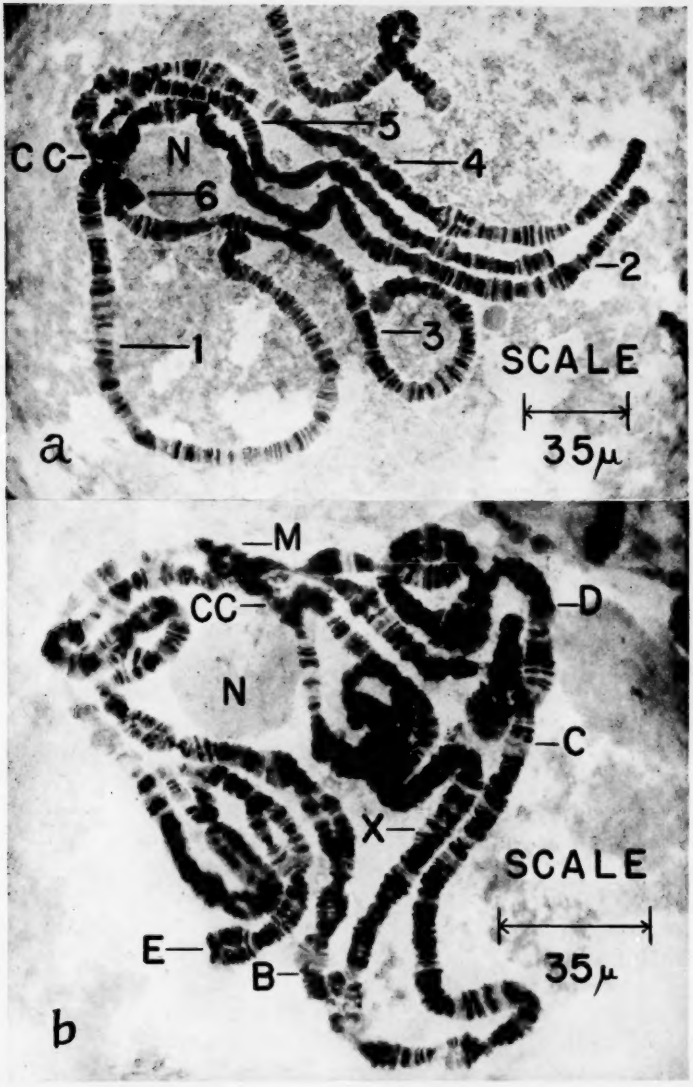


FIGURE 1.—Salivary gland chromosomes (Photomicrographs by MR. MAX FREYDECK). (a) Normal *Drosophila virilis virilis*. (b) Hybrid from the cross *D. virilis virilis*×*D. virilis americana*. The numbers in (a) refer to chromosomes. X, B, C, D, E, and M in (b) refer to salivary gland units in the hybrid. These units correspond to the *virilis* chromosomes 1, 2, 3, 4, 5, and 6, respectively CC=chromocenter; N=nucleolus.

chromatin forming a broad double band in the middle of this area. The heterochromatin in this chromosome is as extensive as that found in the X, a region extending from 42E3 through 42F. In favorable preparations several bands can be detected.

C (3).—The distal end of chromosome 3, like the distal end of chromosome 1, has scattered chromomeres, loosely arranged in the form of bands. Occasionally, too, there is a tendency for the entire end, from 43A1 to 43D1, to be frayed out as in chromosome 1. In region 54E there is a helpful landmark of two double bands and a single band, taking a very intense stain, and typically so close together that they appear as a single very broad band. The conspicuous bulb-like region near the proximal end extending approximately from 60A through 60B also demands special attention. HEITZ has devoted considerable study to this interesting area, an area which is exceedingly difficult to analyze because of the inconsistency of the arrangement of the chromomeres. Occasionally certain bands in this region do not appear as typical cross-striations, but rather as partially ring-like structures. HEITZ suggests that such a structure might result if small pieces were attached like branches to the side of the chromosome in this region. FUJII shows this region as an ill-defined, more or less heterochromatic area. Regions can be seen in various preparations which are in general agreement with either type of structure. On the other hand, in some of my best preparations the bands in this area extend all the way across the chromosome in a perfectly typical manner. In view of this fact, I prefer not to ascribe too much significance to an area which possibly might be just another of those loosely organized, more or less heterochromatic regions encountered not infrequently in other chromosomes. Further study will be necessary before the significance of this region will be definitely known.

Breaks occur with rather high frequency at the weak points near 53C and 57E. With the exception of 6, this is the only chromosome which does not have a conspicuous heterochromatic area at the proximal end.

D (4).—The distal end of chromosome 4 is characterized by sharp, heavy bands. Two weak points, where breaks occur with a very high frequency, are found in this chromosome. These are at 64E and 80B. Two greatly inflated regions, which can be used as landmarks, are located between 68D and 69B, and between 72F and 73C. These regions have rather broad areas of lightly staining diffuse chromatin. The heterochromatic region in the proximal end of this chromosome is equally as extensive as similar areas in the chromosomes 1 and 2. The bands in this area (80E–80F) appear to be much sharper and clearer than similar bands in other chromosomes.

E (5).—The distal end of chromosome 5 can easily be distinguished from

all the other chromosomes by the double, dark band at the extreme tip, and by a similar double band a short distance proximal to the first band, in a region which is usually constricted. The latter band is located at 81D1. An inflated region extending from 84A through 84D is very conspicuous, but variable in shape and staining qualities. A distinctive and useful landmark is shown at 94A. This region is probably made up of two closely apposed double bands. The only definitely weak point found in this chromosome is located in a constricted region at 89A. Another noticeably inflated region is seen between 97D and 98B. The lightly staining chromomeres in this region make it a difficult area to analyze. There is a definite, but not extensive, heterochromatic region at the proximal end of this chromosome. This region, shown at 95F, quite typically has four or five bands that are distinguishable.

M (6).—Chromosome 6, the microchromosome, is only 10–12 micra in length. The distal end appears to be free and not connected with the chromocenter. There is no definite heterochromatic area at the proximal end.

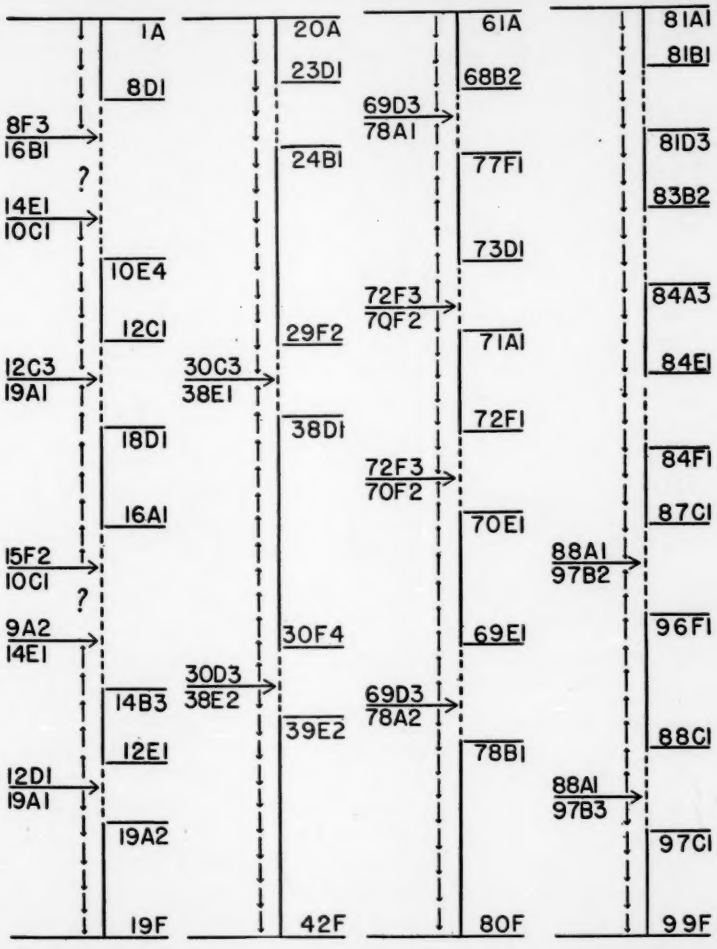
SALIVARY GLAND CHROMOSOMES OF THE NORMAL *D. virilis americana*

Upon superficial examination, the salivery gland chromosomes of *americana* appear to be similar to *virilis*. Just as in the latter sub-species, there are six units, five long and one very short. Moveover, both the proximal and distal ends of the units are similar in the two sub-species. Closer examination, however, reveals striking differences in the linear arrangement of the bands, although there are remarkably few bands, if any, which are not similar in appearance, and perhaps homologous in the two sub-species. Figures 2, 3, 4 and 5 show diagrammatically the arrangement of the bands in *americana* as compared with *virilis* for the X, B, D, and E units. The C and M units are similar in the two sub-species. The differences in the arrangement of the bands are described below in detail.

THE SALIVARY GLAND CHROMOSOMES IN THE HYBRID

The salivary gland chromosomes used for this study were from the cross wild type *americana* ♀ × wild type *virilis* ♂. Salivary gland chromosomes of the hybrid from the reciprocal cross were also examined, as were chromosomes from crosses in which various *virilis* stocks carrying mutations were used instead of the wild type. There were no obvious differences among the hybrids. In the hybrid male, the X is unpaired just as it is in either pure sub-species. In the hybrid there are the same number of salivary gland units as in either pure sub-species, namely: six. In the hybrid, each *virilis* unit has an *americana* homologue, or partial homologue.

Which of the four *virilis* rod-like chromosomes correspond to the two *V*'s (see figure 8 for drawings of ganglion chromosomes) in *americana*



FIGURES 2–5.—Schematic representation of the rearrangement of the bands in the X, B, D, and E units (from left to right) of *D. virilis americana* as compared with *D. virilis virilis*. (See map of the normal *D. virilis virilis* in Plate I.) Broken lines represent regions which have not been observed to pair. Solid lines represent regions which have been observed to pair. Vertical arrows directed downward indicate normal order. Vertical arrows directed upward indicate inverted order. The horizontal arrows indicate approximate position of breaks. The numbers and letters are the same as those used in the map of *virilis*, and indicate the limits of the various regions. Diagram is not drawn to scale.

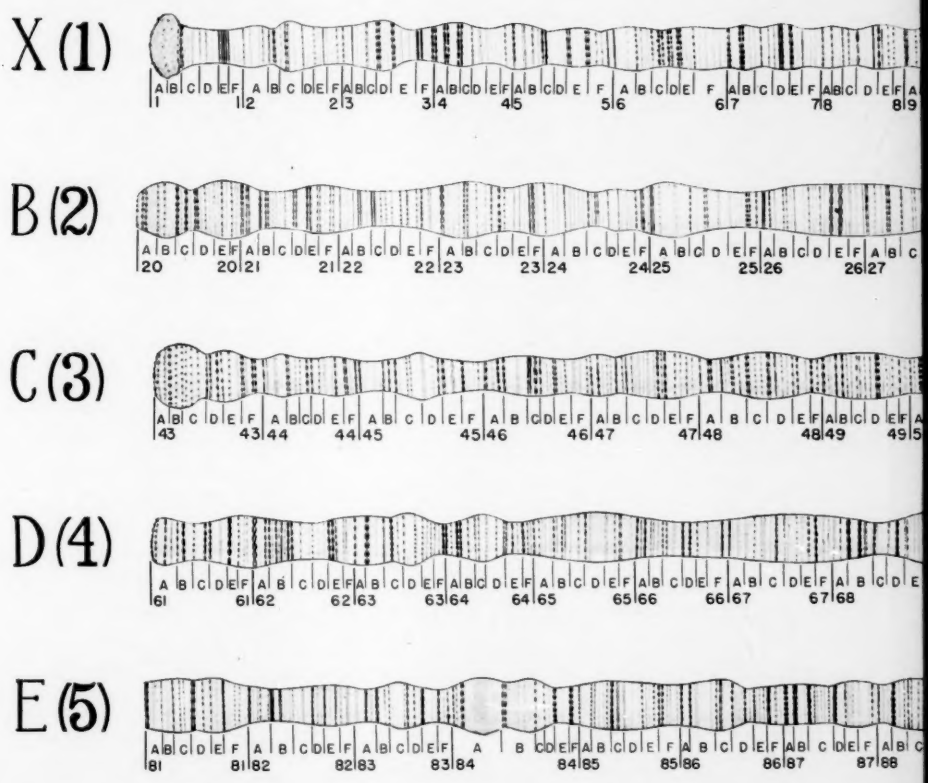
cannot be determined with certainty by direct observation of salivary gland chromosomes, because the arms of the V's appear as distinct units. Since this has not been determined genetically, it is necessary to adopt some convention by which we can avoid the use of the term chromosome when referring to the salivary gland nuclear elements of the hybrid, or of *americana*. The *virilis* chromosomes 1 to 6 are thence designated standard units X, B, C, D, E and M, respectively. This convention will permit us to refer to specific homologues, or partially homologous units regardless of whether these units are arms of a single chromosome, or entire chromosomes. A photomicrograph of the salivary gland chromosomes of the hybrid is shown in figure 1(b).

Unit X (figure 6). The X unit of the female hybrid shows a very extensive difference in the linear order of the bands in the two sub-species. In consequence of this rearrangement of bands, the resulting configurations are sometimes very complicated.

Using the complete pairing of maternal and paternal units as the only safe criterion of homology, the following regions of the *virilis* X unit have homologous regions in the *americana* X unit:

1A through 8D1 (normal order)
10E4 through 12C1 (normal order)
12E1 through 14B3 (inverted order)
16B2 through 18D1 (inverted order)
19A1 through 19F (normal order)

By referring to figure 2, or to the map of *virilis*, it will be seen that the above paired regions do not cover the entire unit. A few bands on either side of practically all the breaks in the order of the bands are without positive homologues. Two other short regions have never been observed to pair in the hybrid, namely: from approximately 9A1 to 10B4, and from 14E1 to 16B1. That a few bands on either side of inversion points fail to pair is not surprising, and may be explained on the simple grounds of opposing physical stresses acting at those points. Presumptive evidence for homology is similarity of banding, that is, similarity of size, position, and staining quality of bands. If such evidence is accepted at its face value, then it can be stated tentatively that, except for the two regions noted above each region of the X unit, band for band, in *virilis* has a homologous region in *americana*. Undoubtedly future studies will reveal complete pairing up to the breakage points for some of these unpaired regions. It seems quite likely that the two remaining sections also have homologues in *americana*. Here again the only supporting evidence for this is the doubtful similarity of the bands. These two sections in *americana*, although of considerable length, have never been observed to pair with


X (1)
B (2)
C (3)
D (4)
E (5)

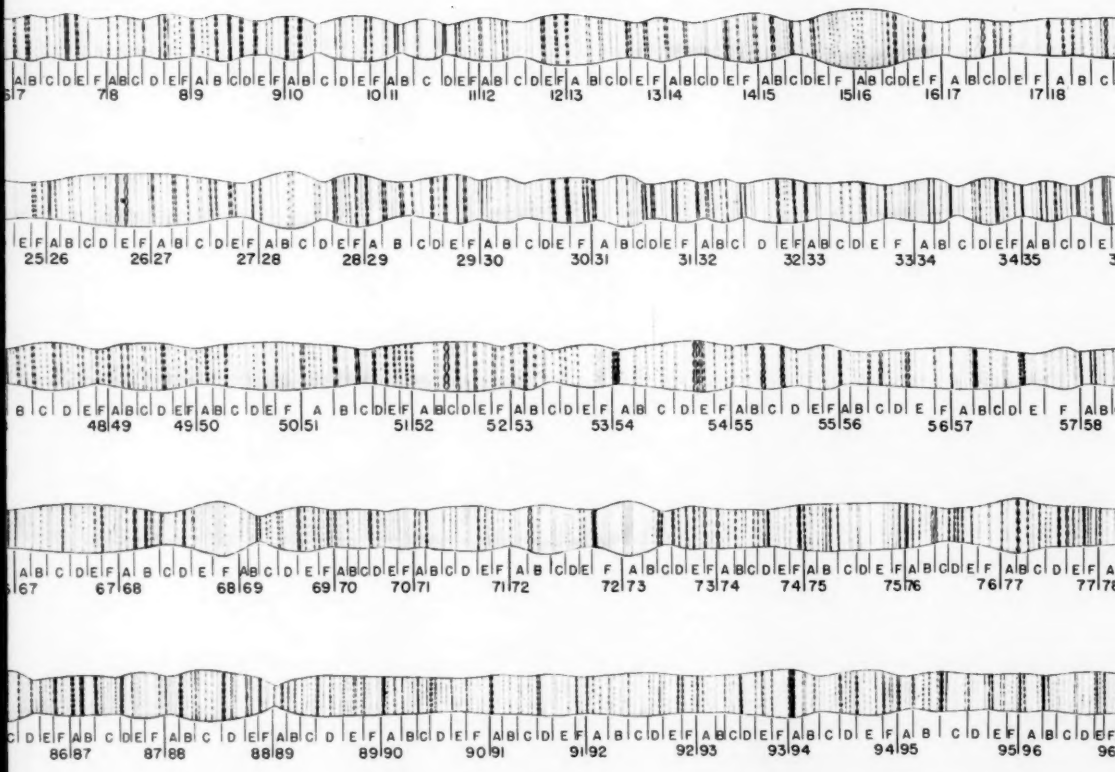
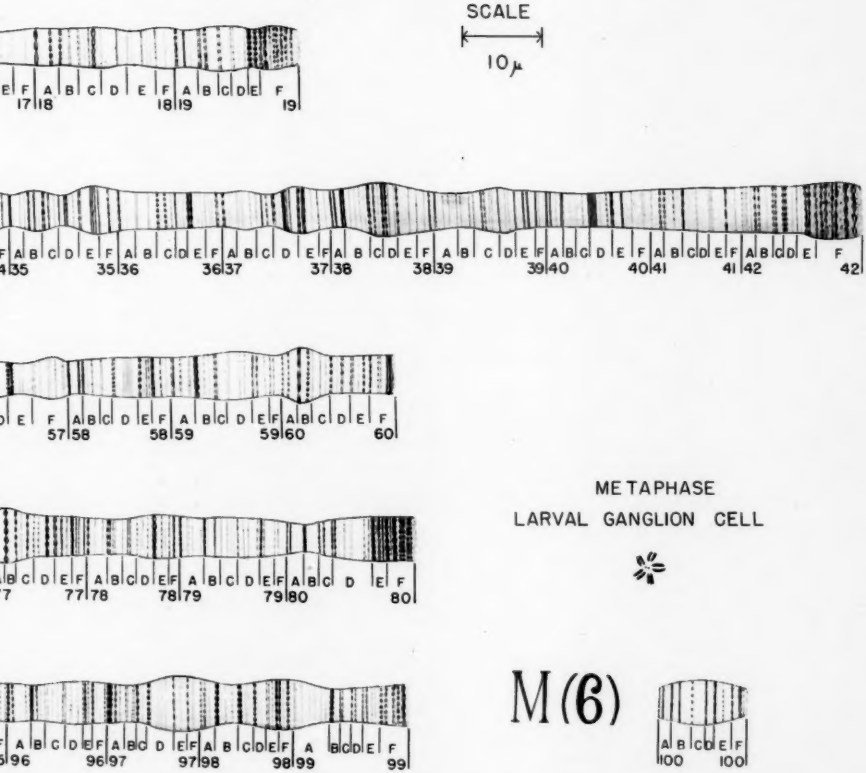


PLATE I.—Map of the normal salivary gland chromosomes of *Drosophila virilis virilis*.


SCALE
10 μ
E F A B C D E F A B C D E F
17 18 18 19 19
F A B C D E F A B C D E F A B C D E F A B C D E F A B C D E F A B C D E F A B C D E F A B C D E F A B C D E F
35 35 36 36 37 37 38 38 39 39 40 40 41 41 42 42
E F A B C D E F A B C D E F A B C D E F A B C D E F
57 58 58 59 59 60 60
B C D E F A B C D E F A B C D E F A B C D E F A B C D E F
77 78 78 79 79 80 80
A B C D E F A B C D E F A B C D E F A B C D E F A B C D E F
96 96 97 97 98 98 99 99
METAPHASE
LARVAL GANGLION CELL
M(6)
A B C D E F
100 100

similar regions in *virilis*. This possibly is because they are so greatly displaced relative to their doubtful homologues in the other sub-species. The resulting competition in pairing with other sections might then be too great. Even known homologous regions are not usually paired simultaneously in a single nucleus.

Additional evidence bearing on the complete homology of the X units in the two sub-species was obtained from the following crosses. *Virilis* males carrying various combinations of the sex-linked mutant genes: sepia, yellow, scute, echinus, crossveinless, vermilion, singed, forked, white, apricot, and ragged, were crossed with wild type *americana* females. All hybrids were wild type showing that for each locus tested there is a wild type allele in *americana*. Since the tested loci have not been located on the salivary gland chromosome map, some of them may or may not be located in the regions 9A1 to 10B4, and from 14E1 to 16B1. Therefore for the present it seems safest to consider these two regions in *virilis* as having doubtful homologues in *americana*.

The order of the bands for the X unit in *americana* is distinctly different from that of *virilis* (figure 2). Assuming that the doubtful regions mentioned above are homologous, or partially homologous, then there are six interruptions in the sequence of bands in the hybrid. Such differences in sequence can possibly be accounted for by inversions in the X unit of the ancestral form common to both sub-species, since only transposition of bands is involved.

Unit B (figure 6). The B unit shows one simple inversion in the hybrid. Typically there is rather close pairing of the two homologues throughout their length with the exception of a small region from 23D to 24B, and short variable regions at either end of the inversion points. Even in those regions not yet observed to undergo pairing, there appears to be complete similarity of bands in the two homologues. Therefore it may be tentatively stated that there is complete homology of bands for this unit in the two sub-species, and that the two sub-species differ from one another only by a simple inversion.

The following regions have been observed to pair in the hybrid:

20A through 23D1 (normal order)
24B1 through 30A1 (normal order)
30E4 through 38D1 (inverted order)
39E1 through 42F (normal order)

The distal end typically exhibits less complete pairing, even in known homologous regions, than does either the middle, or proximal portions. The order of bands is schematically shown in figure 3, for the B unit of *americana*.

Unit C. In the C unit the *americana* and *virilis* elements appear to be completely homologous throughout their lengths, and without any re-

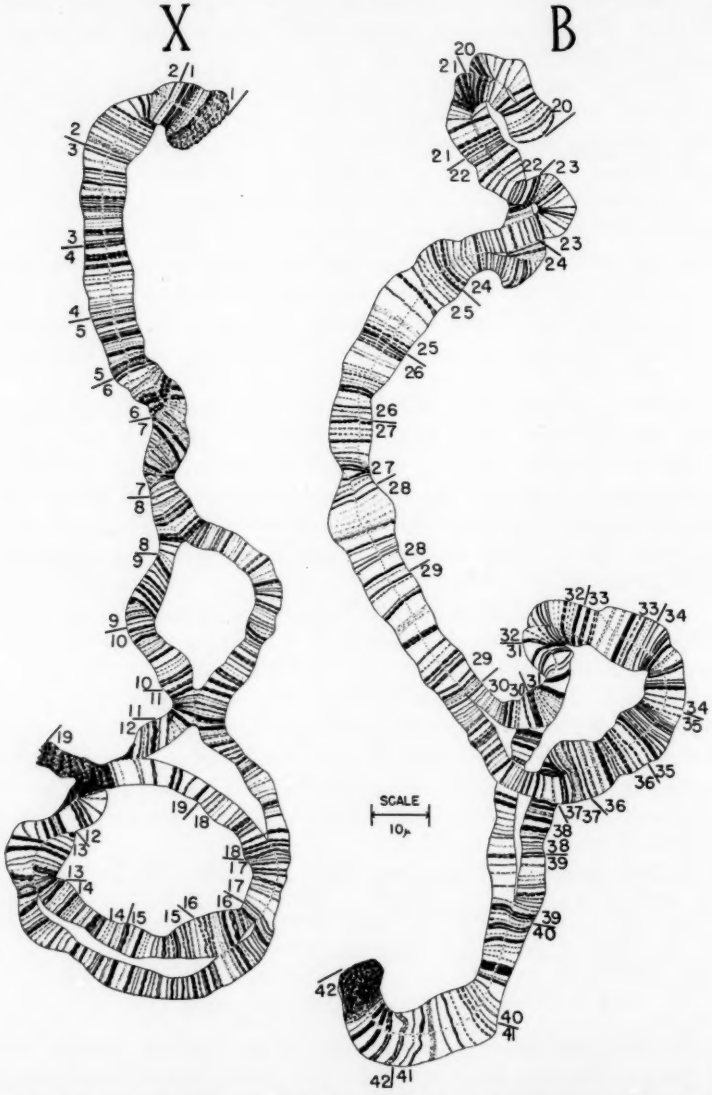


FIGURE 6.—Camera lucida drawings of the X unit and B unit of the salivary gland nucleus of the hybrid from the cross *D. virilis virilis*×*D. virilis americana*.

arrangement in the sequence of the bands. Except for the small M chromosome, this is the only unit of the entire set which does not show some major

FIGURE 7.—Camera lucida drawings of the D unit, E unit, and M unit of the salivary gland nucleus of the hybrid from the cross *D. virilis virilis*×*D. virilis americana*.

rearrangement of the bands in *americana* as compared with *virilis*. Typically pairing is almost as complete for this unit as in either pure sub-

species. There is a tendency for pairing to be incomplete rather frequently, however, from approximately 45A to 45E, and from approximately 55E to 56E. In both of these sections pairing has been observed several times. Occasionally the three broad bands in *virilis* in region 45B are slightly different in appearance from corresponding bands in *americana*. The peculiar region in the proximal end at 60A–C, appears to be homologous in the two sub-species.

Unit D (figure 7). Complete pairing has been observed in the following regions:

61A through 68D1 (normal order)
69E1 through 70E1 (inverted order)
71A1 through 72F2 (normal order)
73C1 through 77F1 (inverted order)
78B1 through 80F (normal order)

The bands in the short non-paired regions on either side of the breaks appear to be definitely similar, and are probably homologous in the two sub-species. The arrangement of the bands in *americana* could have been derived from an arrangement like that in *virilis* by assuming that a long section became inverted followed by a reinversion of part of this section. The order of bands in *americana* is represented schematically in figure 4.

Unit E (figure 7). The E unit is characterized by a simple, long inversion extending from 88A2 to 97B3. The regions which have been observed to pair one or more times are as follows:

81A1 through 81B1 (normal order)
81D3 through 83B2 (normal order)
84A3 through 84E1 (normal order)
84F1 through 87E1 (normal order)
88C1 through 92F1 (inverted order)
93C1 through 96C1 (inverted order)
97C1 through 99F (normal order)

The pairing in this unit is typically much looser than in any of the other units, and the homologous regions tend to pair only in small sections, especially in the distal end; perhaps the distal end of this unit should thus be suspected of harboring non-homologous bands in the hybrid. Figure 5 shows the order of bands for *americana*.

Unit M (figure 7). Typically the M unit is unpaired, or loosely paired in the hybrid. In some few figures, however, pairing has been observed for practically the entire length of this unit. At present there is some doubt as to the homology of the bands in the distal region of this microchromosome, although even in this region they appear to be similar.

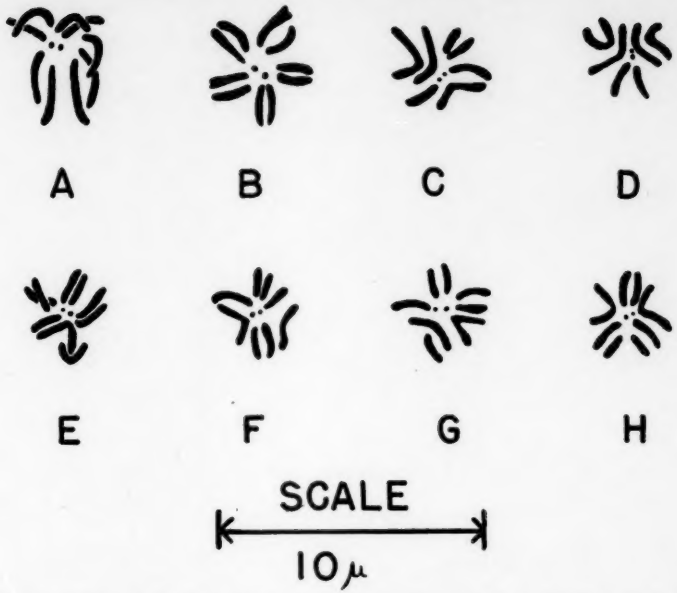


FIGURE 8.—Camera lucida drawings of metaphase configurations from larval ganglion cells.
(A) male, and (B) female *D. virilis virilis*.
(C) male, and (D) female *D. virilis americana*.
(E) male, and (F) female hybrids from the cross *D. virilis americana* male×*D. virilis virilis* female.
(G) male, and (H) female hybrids from the cross *D. virilis virilis* male×*D. virilis americana* female.

THE CHROMOSOMES OF THE GANGLION CELLS

The metaphase plates of the ganglion cells from the larvae or pupae of male and female *D. virilis virilis* show five pairs of rod-shaped chromosomes of approximately equal size, and one pair of microchromosomes (figure 8, A and B). Because of the ease with which *americana* can be crossed with *virilis*, it was a distinct surprise to discover that the metaphase plates of the ganglion cells of *D. virilis americana* have two pairs of V-shaped chromosomes, one pair of rods, and a pair of microchromosomes in the female, and the same configuration for the male except that one of the V's is replaced by two rods (figure 8, D and C). In the male typically two of the rods show somatic pairing with the single V. The expected configurations are found in the hybrids. Thus both male and female hybrids from the cross *virilis* ♂×*americana* ♀ show two V's, six rods, and a pair of microchromosomes (figure 8, G and H). Configurations of the hybrids from the reciprocal cross show one V, eight rods, and a pair of micro-

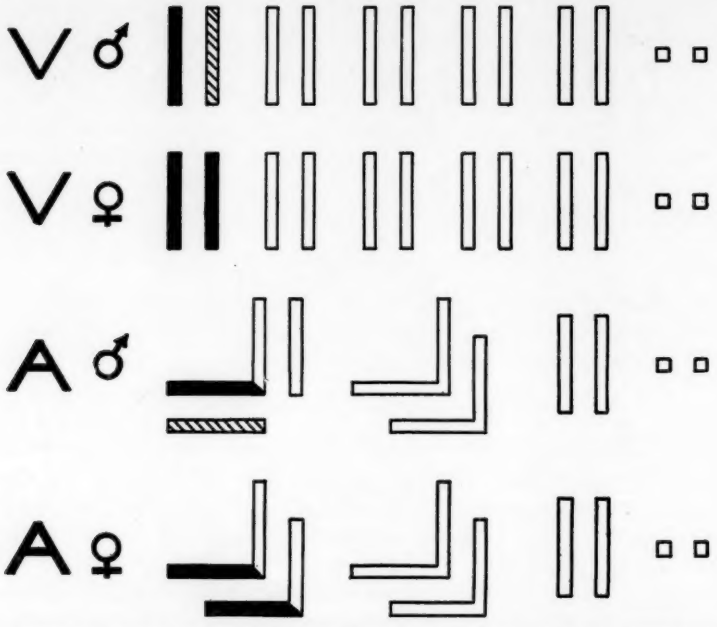


FIGURE 9.—Diagram showing the relation of the X and Y chromosomes to the autosomes in the two sub-species *D. virilis virilis* and *D. virilis americana*. The X chromosome is solid, the Y chromosome is cross hatched, and the autosomes are in outline. V=*virilis;* A=*americana*

chromosomes for the male, and two V's, six rods, and a pair of microchromosomes for the female (figure 8, E and F).

Study of the configurations in the hybrid from *americana* ♂ × *virilis* ♀ and in the pure *americana* shows that the male has one less V-shaped chromosome than the female, and that the male has two additional rods not seen in the female, that is, one of the V's of the female is replaced by two rods in the male. Therefore, in terms of *virilis* chromosomes, the X of *americana* is fused with one of the autosomes, while the Y remains separate. The relationship of the X and Y chromosomes to the autosomes in the two sub-species is represented diagrammatically in figure 9. In terms of *virilis* chromosomes it is not known at present which autosome is fused to the *americana* X unit to form one of the V-shaped chromosomes, or which two chromosomes of *virilis* are fused to form the other V of *americana*.

DISCUSSION

The closeness of relationship between the two sub-species *virilis* and *americana* is shown by the comparative ease with which they may be

crossed, the partial fertility of the hybrids (rare for Drosophila hybrids), and more particularly by the similarity of the banded structure of the salivary gland chromosomes. In fact, except for the arrangement of the bands, it is at present doubtful whether or not there are any appreciable differences between them. Even with respect to the linear order of the bands, it appears that the rearrangement can be accounted for by inversions alone. That is, in the hybrid there are no known translocations or duplications, and, with the possible exception of the two short sections in the X unit, no known non-homologous regions. The differences between the banded structure of the salivary gland chromosomes in the two subspecies is then of the same kind, and not greatly different in degree from the differences found among the various strains of *pseudoobscura* (DOBZHANSKY and STURTEVANT; DOBZHANSKY and QUEAL 1938). The various strains of *pseudoobscura* cannot be distinguished phenotypically one from each other, which is in striking contrast to the morphological and physiological differences between *virilis* and *americana*. It follows then, at least for the two examples noted above, that the phenotypic differences between two sub-species, are not necessarily dependent upon the *amount* of reshuffling of genes as brought about by inversions. That inversions *per se* generally do not cause any phenotypic changes is well substantiated by the many experiments on laboratory stocks known to be genetically similar except in one or more inversions. In certain instances, on the other hand, phenotypic differences are known to follow from transposition of genes, for example: the well known Bar case (BRIDGES 1936), and phenotypic changes following inversions (EMMENS 1937, and others). The above considerations bring up the question of the relative roles played by the various mechanisms in the phenotypic differentiation of groups of Drosophila. Insufficient evidence is presented in the present paper to answer this question, but a comparison of *virilis* and *americana* with other Drosophila groups which have been hybridized is of interest.

Among the mechanisms which are known to effect changes in morphological and physiological expression of characters in various groups of Drosophila, and thus serve to differentiate them phenotypically are the following: (1) position effect, (2) gene mutations, and (3) quantitative changes in euchromatin. Potentially, at least, two other mechanisms may come under the same category as those above, namely (4) quantitative changes in the heterochromatin, and (5) changes in kinetochores.

Our understanding of position effects has been derived from studies of rearrangement of genes in Drosophila stocks of presumably the same strain and not from hybridization experiments. That the latter type of experiments may be useful in this connection may be indicated by the correlation, (or the lack of it) between extent of rearrangements and phenotypic

divergence of the parents in certain hybrid crosses. SPENCER states that in his opinion (written communication) in the morphological and physiological differentiation of the parents the following seriation can be made for the five groups of hybrid crosses listed below: (The pair is placed first in which the parents are most similar.)

(1) *pseudoobscura*—Race A–Race B
(2) *pseudoobscura—miranda*
(3) *azteca—athabasca*
(4) *virilis—americana*
(5) *melanogaster—simulans*

On the basis of the number and kinds of rearrangements found in the euchromatic portions of the salivary gland chromosomes of the hybrids, the ranking of the same groups would seem to be as follows: (greatest similarity first)

(1) *melanogaster—simulans* (PÄTAU; HORTON)
(2) *pseudoobscura*—Race A–Race B (DOBZHANSKY and STURTEVANT)
(3) *virilis—americana*
(4) *pseudoobscura—miranda* (DOBZHANSKY and TAN)
(5) *azteca—athabasca* (BAUER and DOBZHANSKY 1937)

In so far as the above limited comparisons offer valid data for generalization, then it may be concluded that morphological and physiological differentiation does not necessarily accompany even extensive diversity in the linear order of the salivary gland chromosome bands.

The sub-specific differences between *virilis* and *americana* may be attributed to gene mutations. This seems probable since SPENCER suggests that his preliminary studies of the two sub-species indicate that these differences depend upon multiple factors. This, of course, does not rule out the possibility that other mechanisms are at least partly responsible for the sub-specific characters. Further data will have to be accumulated before the role played by gene mutations can be definitely determined, both for these two sub-species and other groups of Drosophila which have been hybridized.

It is obvious, of course, that the addition or loss of euchromatic material is capable of producing drastic changes in the phenotype. However, it does not follow that the addition or loss of euchromatin always produces extensive morphological changes. Compare, for example, hybrids of *virilis americana* and *melanogaster-simulans* with the hybrids from the crosses *pseudoobscura-miranda* and *azteca-athabasca*. The hybrids from the former two crosses show only minute, if any, non-homologous regions (therefore the loss or addition of euchromatin has been negligible), whereas the hybrids from the latter two crosses show many non-homologous regions.

Since the heterochromatin (HEITZ), when present, is an integral and

apparently constant part of a salivary gland chromosome, it must be taken into account in any consideration of the possible mechanisms controlling the morphology and physiology of the phenotype. Indeed the location of the gene for bobbed in the heterochromatic region of the X chromosome and the discovery of an homologous region in the Y chromosome which can be detected in the salivary gland nucleus (PROKOFYEVA-BELGOVSKAYA 1937), show that heterochromatin in this instance at least, is not inert. Y chromosomes in Drosophila are generally heterochromatic in certain phases of the meiotic cycle, and either are not detected in the salivary gland cells, or the portions which appear there are heterochromatic also (for example, *melanogaster*). For the most part, it appears that Y chromosomes are truly inert and exert no effect on the phenotype. For example, in spite of the wide variations in the size and shape of the Y chromosome of the different strains of *pseudoobscura*, the strains are practically indistinguishable (DOBZHANSKY 1935). However, at the present time, it is unsafe to generalize about the effectiveness of heterochromatin in producing phenotypic changes.

The interesting possibility that kinetochores *per se* may be agents capable of effecting phenotypic changes apparently has not been subjected to analysis. The present study unfortunately does not throw any light on this possibility, but it may be pointed out here that we have in the two subspecies *virilis* and *americana* unusually favorable material for elucidating this problem, for it is evident that during the course of evolution these two sub-species have undergone great changes with respect to the kinetochores, some of which may have effected changes in the phenotype. Moreover, the partial fertility of the hybrid not only will make it feasible to test the possible role of the kinetochores, but also the other mechanisms discussed above, namely: position effect, mutations, and quantitative changes in the euchromatin and heterochromatin.

The study of the salivary gland chromosomes of these two species is in strict agreement with similar studies of Drosophila hybrids in showing that the inversion is by far the most common type of chromosomal change found in natural populations. Regardless of whether there are few or many rearrangements in the hybrid, and regardless of the rank of the parents, inversions predominate. The reasons for the absence, or rarity of translocations in wild populations have been discussed by STURTEVANT and BEADLE (1936), and others.

A further consideration of inversions is of interest. Inversions which occur in V-shaped chromosomes almost invariably are confined to the arms, and do not involve the kinetochore. In other words, in the differentiation of Drosophila species and races, the rearrangement of the chromosomal material is generally confined to the salivary gland units, that is,

elements of the salivary gland nucleus whether these are entire chromosomes corresponding to the rod-shaped chromosomes of the gonial cells, or arms of V-shaped chromosomes. How general is this characteristic behavior of blocks of genes to act as units (though the constituent parts may be variously reshuffled) in the differentiation of species may be seen in a study by STURTEVANT (1938). He has been able to show that *virilis*, *miranda*, *pseudoobscura*, *simulans*, and *melanogaster* have comparable blocks of genes (salivary gland units) which are essentially homologous, and which have behaved as units during the course of the evolutionary history of the species in that there has been no transference of large numbers of genes from one unit to another. The relative stability of the salivary gland units (in the sense described above) has resulted in what appears to be a rather general occurrence of six units in Drosophila species. One notable exception to this rule is found in *D. azteca* (DOBZHANSKY and SOCOLOV 1939) which has seven. This is accounted for on the assumption that a translocation has been involved during the formation of this species.

Into the arena of the controversy centering around the question of what constitutes a species one enters with great hesitancy. Yet the present study, in my opinion, does offer several relevant data, which, in conjunction with the facts brought out by similar studies, may contribute in some small degree to our better understanding of this question.

The accepted criterion for the assigning of a definite taxonomic position to organisms is the genetic relationship of the organisms in question. In practice genetic relationship is of necessity a determination, or more correctly, an assumption based usually on morphological characters alone. As pointed out above, on the basis of morphological differences alone *virilis-americana* stands near the bottom of the list, that is, the sub-species *virilis* and *americana* are more dissimilar than are, for example, species *pseudoobscura* and *miranda*, or species *azteca* and *athabasca*. When the extent of the rearrangements found in the banded structure of the salivary gland chromosomes for the same three pairs is considered, there can be little doubt that *virilis* and *americana* are more closely related. When ganglion configurations are considered, *virilis* and *americana* are definitely the most dissimilar of any of the Drosophila species which have been hybridized, although such dissimilarities are of doubtful value in establishing genetic relationship. Sexual isolation, hybrid fertility, and other factors can be made the basis for similar comparisons.

The few examples above show that no simple formula is available for the establishment of the taxonomic position of a new organism, even when the factors which determine the genetic relationship are known. In the last analysis, it becomes necessary to arbitrarily evaluate the relative im-

portance of the dissimilarities between any two groups which may be hybridized.

The striking differences between the metaphase configurations of the ganglion cells of *virilis* and *americana* demands special attention. No other two sub-species of the genus Drosophila are known to differ so much. A consideration of these differences alone would lead one to infer that the two sub-species are widely different genetically also. Such an inference is, of course, unjustified in view of the many factors pointing to their rather close relationship. Among these factors may be listed the following: comparative ease with which they can be crossed; viable and vigorous hybrids which can be crossed *inter se*, or backcrossed to either parent sub-species; wild type alleles in *americana* for all *virilis* mutations tested; and notably few regions in the salivary gland chromosomes which are even doubtfully non-homologous. The obvious conclusion to be drawn from the above considerations is that the true genetic relationship of two sub-species, or species, cannot be judged solely by the metaphase configurations of the gonial, or ganglion cells. This conclusion is proved in the converse way by studies of hybrids from the cross *pseudoobscura*×*miranda* (DOBZHANSKY and TAN 1936). The females of these two species have the same configuration in the öogonial cells, yet the salivary gland chromosomes of the hybrid show extensive rearrangements of the bands, and many non-homologous regions. These differences are much greater than in the cross *virilis* ×*americana*. In addition, the hybrids are completely sterile, or, under certain conditions the female is only slightly fertile (MACKNIGHT 1939).

Another point of great theoretical interest is brought to the fore by the consideration of another aspect of the metaphase configurations. It is obviously unlikely that two sub-species as closely related as *virilis* and *americana* could represent convergent types having in common only a very remote ancestor. The common ancestor must have had a metaphase configuration which could be resolved into either the *virilis* type or the *americana* type rather simply by the attachment of rod-shaped chromosomes to form V's, or the disjunction of the arms of the V's to form rod-shaped chromosomes. The questions therefore arise: Is one of the sub-species more primitive than the other? Or did both sub-species arise from an ancestor which was intermediate with respect to the chromosome configuration? Conceivably any one of these three possibilities might obtain, but at present there is insufficient evidence to favor one possibility over the others. To whichever type the ancestor conformed, it is unlikely that the divergence between *virilis* and *americana* was accomplished in a single step. If the disjunction of arms of V's to form rods, or the attachment of rods to form V's, were common, it seems that such occurrences would

have been observed more frequently in the extensive investigations of Drosophila.

It is reasonable to suppose that the disjunction of the arms of two V's to form four rods, or the attachment of four rods to form two V's, did not occur simultaneously. Therefore, at least one of the sub-species, and possibly both, very probably had an ancestor whose ganglion chromosome configuration was intermediate between *virilis* and *americana*, that is, an ancestral form having one pair of V's, three pairs of rods, and a pair of microchromosomes. It would be of interest if such a form were found still to exist in nature.

No matter whether the change from V's to rods, or vice versa, was accomplished in one or several steps, the fate of the kinetochores is of great importance. Geneticists and cytologists alike consider the kinetochore to be a rather stable element of the chromosome. Yet in the formation of these two sub-species it is apparent that the kinetochores have undergone changes as drastic and far-reaching as the banded structure of the chromosomes, if indeed not more so. Let it be assumed, for instance, that *americana* has been derived from an ancestor whose chromosome configuration conforms to the *virilis* type, that is, a type having only rods and no V's. If such were the case, it is evident that kinetochores have been lost or have fused. The only alternative to such a view is the possibility that the V's derived from two rods retain both kinetochores. On *a priori* grounds there is no more reason to suppose that there are two kinetochores in the V's of *americana* than in the V's of *melanogaster*. If kinetochores have been lost in the formation of the V's of *americana*, then the loss must have been confined to the kinetochore region without involving any part of the banded structure as seen in the salivary gland nuclei. As previously pointed out, the proximal region of each unit in *americana*, apparently including the heterochromatin, definitely has a homologue in *virilis*. This is not to imply that a break immediately distal to the kinetochore, with the loss of the kinetochore could not take place. On the contrary, it is reasonable to suppose that a chromosome sustaining a break in the kinetochore region will have a greater survival value in the homozygous condition than a chromosome sustaining a break in a more distal region. Unfortunately the kinetochore has not been satisfactorily identified in the salivary gland chromosomes, and the loss of one, or the presence of two would go undetected.

In this connection it is interesting to recall that PAINTER and STONE (1935), in their study of certain "fusions" between chromosomes X and Y in *D. melanogaster*, suggest that there are three main types of "fusions." One type probably involves the actual fusion of the kinetochores of two chromosomes. The other two types of "fusions" involve the loss of the kinetochore from one of the chromosomes. In the latter two types, the chromo-

some which has lost its kinetochore becomes attached, or fused with the other at a point just distal to the kinetochore. This kinetochore will now do double duty. There is no evidence that any one of these three types of fusions have played a role in the formation of the *americana* configuration, but such a possibility in not excluded.

If it is assumed that *virilis* is derived from an ancestor which had V-shaped chromosomes with a single kinetochore, we are confronted with the problem of how kinetochores arise. Since the evidence is overwhelmingly against the view that kinetochores arise *de novo*, some other hypothesis must be postulated. One not improbable explanation is the possibility of obtaining a division of a single kinetochore to form two, with their disjunction unaccompanied by division of the chromosomes. Another is the possibility of an actual split occurring in a kinetochore, dividing the single kinetochore into two functional parts as McCLINTOCK (1932) has described for the formation of functional ring-shaped chromosomes in maize following X-ray treatment.

The fate of the kinetochores in the evolution of these two sub-species is obviously of great interest since a solution of the questions involved should throw light on the physiology of the chromosomes in general. I am at present undertaking a further investigation of this feature in the two sub-species.

SUMMARY

1. A new map of the salivary gland chromosomes of *D. virilis virilis* is presented.

2. Both male and female hybrids from the cross *virilis*×*americana* are partially fertile when crossed *inter se*, or backcrossed with either parent.

3. A study of the salivary gland chromosomes of the hybrids reveals that the two sub-species *virilis* and *americana* differ by a number of rearrangements. Four of the six units are affected. All of the rearrangements can be accounted for by inversions except possibly in the X unit, which has two short sections which may be non-homologous.

4. The metaphase configurations of the ganglion cells in the two subspecies are distinctly different. Both male and female *virilis* have six pairs of chromosomes, five pairs of rods and one pair of dots. The *americana* female has four pairs of chromosomes, two pairs of V's, one pair of rods, and one pair of dots. The *americana* male differs from the female in having one of the V's replaced by two rods.

5. A study of the metaphase configurations shows that in terms of the *virilis* chromosomes the X chromosome of *americana* is fused with one of the autosomes, while the Y remains separate.

6. A comparison of *virilis* and *americana* with other Drosophila groups which have hybridized is made.

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CORRIGENDA

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Volume 23

Page 520, paragraph 3, line 5, for "state" read "stage."
Page 600, explanation of figures 7–17, line 5, figures 1, 7, 8, 9, 5, 2, 3, 4, 6,10 should read 7, 13, 14, 15, 11, 8, 9, 10, 12, 16 respectively.

Volume 24

Page 310, sixth line from bottom of page, for "justificable" read "justifiable."
Page 366, paragraph 3, line 2, for "deficiences" read "deficiencies."
Page 368, footnote 2, line 2, for "P^{wr}" read "P^{ww}."
Page 377, paragraph 3, line 4, for "*M-M ms*" read "*M-R ms*."
Page 429, line 4, for "GOWFN" read "GOWEN."
Page 432, table 14, bottom left, for "y^1z" read "y^4z."

EDWARD MURRAY EAST

In the death of EDWARD MURRAY EAST at Boston, Massachusetts, November 9, 1938 genetics has lost one of its best known pioneers and leaders. A careful worker, a keen thinker, a scholarly writer and able lecturer, he will be remembered by his students and colleagues also as a wise counselor and friend.

In considering the life of any man of science, especially a geneticist, it is proper to look over his family tree to hunt out any germs of genius that might have been his inheritance. There is a tradition in the East family, never fully proved, that SIR ISAAC NEWTON was numbered among the collateral ancestors. Whether or not this is true, the family must have admired NEWTON for the name recurs in several generations. Better substantiated is the family history that one of his ancestors was a former Jesuit priest who had been excommunicated for his scientific beliefs, came to America in 1705, married and had three grandsons who fought in the American revolution. His mother's immigrant paternal ancestor was MATTHEW WOODRUFF who in 1640 helped establish Farmington, Connecticut. A great uncle, EBENEZER BUSHNELL, was a Congregational minister and administrative officer in Western Reserve University. His father, WILLIAM HARVEY EAST was graduated from the University of Illinois as a mechanical engineer and later was a manufacturer of machinery. He married SARAH GRANGER WOODRUFF and their son EDWARD was born October 4, 1879 at Duquoin, Illinois.

With such a background it is not surprising that young EDWARD EAST should have been interested in study and in science. The mental energy exhibited in his later life was anticipated in a precocious childhood. At an unusually early age he was reading the classics; at fifteen he was graduated from high school. Partly on account of family interest in Cleveland he entered the Case School of Applied Science in 1897 but after one year transferred to the University of Illinois where he received the B.S. degree in 1900. After graduation he was employed as assistant chemist in the Illinois Agricultural Experiment Station working with DR. C. G. HOPKINS in his selection experiments to alter the chemical composition of corn. He was joint author with C. G. HOPKINS and L. H. SMITH in publications dealing with "The Structure of the Corn Kernel and the Composition of Its Different Parts," and "Directions for the Breeding of Corn, Including Methods for the Prevention of Inbreeding," published as bulletins of the Illinois Agricultural Experiment Station. In 1903 DR. EAST was made first assistant in plant breeding. He also carried on graduate study in the University and received the M.S. degree in 1904 and Ph.D. in 1907. When the Adams Fund was made available to the state experiment stations by the Federal Government, he was employed by the Connecticut Agricultural Experiment Station and moved to New Haven in September 1905. His doctoral thesis was based upon "A Study of the Factors Influencing the Improvement of the Potato" published in 1908 as a bulletin of the Illinois Agricultural Experiment Station.

In the investigations dealing with the alteration of the composition of the corn kernel DR. EAST soon realized the importance of inbreeding. Unable to interest his chief in this problem he self-fertilized a number of plants of the Leaming variety in 1904 on his own initiative. This inbred material was brought to Connecticut and served as the beginning of an intensive study of the effects of inbreeding and crossing.

Following the clear statement of DR. GEORGE H. SHULL in 1908 outlining the importance of inbreeding in isolating desirable germ plasm and of crossing to secure maximum vigor and productiveness, DR. EAST secured additional evidence which proved the value of this method of corn improvement and in this investigation, started independently, he developed basic information for a

revolutionary system of seed corn production. As collaborator with the Bureau of Plant Industry in the United States Department of Agriculture he published in bulletin form "Heterozygosis in Evolution and in Plant Breeding." Later these investigations were brought together and summarized in a book, "Inbreeding and Outbreeding."

DR. EAST'S major interests from the start were with fundamental principles of heredity. The early investigations helped to place Mendelism on a firm foundation and his interpretation of the Mendelian inheritance of quantitative characters based upon duplicate factors was the first clear presentation of this important analysis. He formulated and his experiments contributed greatly toward the establishment of the laws underlying the inheritance of size characters. This conception, now accepted by biologists and known as the multiple factor theory, brought heredity under one consistent principle. The publications in the American Naturalist, 1910 and 1911: "A Mendelian Interpretation of Variation that is apparently Continuous" and "The Genotype Hypothesis and Hybridization" are his principal contributions to the solution of this problem.

Largely due to the recommendation of PROFESSOR WILLIAM BATESON, who had given the Silliman Lectures at Yale University shortly before, DR. EAST was appointed to the staff of the Bussey Institution of Harvard University in 1909 where he was made Professor of Experimental Plant Morphology in 1914 and Professor of Genetics in 1926. Here he centered his major interest on the genus Nicotiana, adding to knowledge of the inheritance of quantitative characters and of cross and self-sterility. At the time of his death he had nearly completed a monograph on self-sterility in families of flowering plants.

The years at the Bussey Institution were productive of numerous scientific papers, books, and lectures given at the University of Chicago in 1911, at the University of Missouri in the Graduate School of Agriculture in 1914, Scripps Institution 1920, Johns Hopkins University 1922, Cornell University 1922, Yale University 1924, 1925, Kenyon College 1927, University of Michigan 1930, New York University 1931. In 1926 he received the honorary LL.D. degree from Kenyon College.

During the war he was chairman of the Botanical Raw Products Commission and member of the Botany and Agriculture Commission of the National Research Council and acting chief of the statistics division of the United States Food Administration. At the Institute of Politics at Williamstown he served as chairman of the round table on Population Problems. He was a member of many scientific societies including the American Academy of Arts and Sciences, The National Academy of Sciences and the American Philosophical Society. He was President of the American Society of Naturalists in 1918, honorary Vice-President of the Sixth International Botanical Congress in 1935 and President of the Genetics Society of America in 1937.

In addition to his work in plant genetics, he was interested in the application of genetics to human welfare. His book, "Mankind at the Crossroads" has been published in several foreign languages. "Heredity and Human Affairs" and "Biology in Human Affairs," the latter a collection of articles which he edited, dealt with the application of genetics to economic and social problems.

As a teacher he has had perhaps his greatest influence. For thirty years students from the United States and foreign countries came to DR. EAST for instruction and stimulus. A member of the Editorial Board of Genetics since its beginning he has a well-earned place in the field of genetics.

Our portrait of DR. EAST is reproduced from a photograph by ANDRE SNOW of Brookline, Massachusetts, taken in December 1935.

DONALD F. JONES

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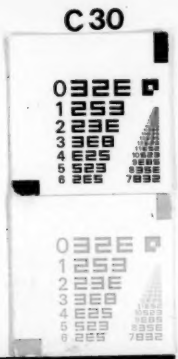
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